DISSERTATION

EVALUATION AND RESOLUTION OF TWO SAMPLING METHODS FOR AIRBORNE AROMATIC DIISOCYANTE MONOMERS

Submitted by

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ABSTRACT

EVALUATION AND RESOLUTION OF FIELD AND LABORATORY DESORBED SAMPLE METHODOLOGIES FOR AIRBORNE AROMATIC DIISOCYANATE MONOMERS

Isocyanates are highly reactive electrophilic compounds used extensively for the production of a wide range of polyurethane end-use products that are indispensable to a number of industries, and also in our everyday life. Characterized by two NCO functional groups attached to an aliphatic or aromatic parent compound, isocyanates are low molecular weight compounds. Due to their reactivity, isocyanates are potent sensitizers and the leading cause of occupational asthma. Other isocyanate exposure-related health effects, such as acute irritation, bronchial hyperactivity, contact dermatitis, and hypersensitivity pnuemonitis are well recognized. The Occupational Safety and Health Administration (OSHA) has promulgated a set of ceiling Permissible Exposure Limits (PELs) of 20 parts per billion (ppb) for the most used aromatic isocyanates, methylene bispenyl isocyanate (MDI) and toluene diisocyanate (TDI). To measure airborne isocyanates, the Occupational Safety and Health Administration prescribes the use of a reagent-coated glass fiber filter that is sent to a laboratory after collection for extraction and then HPLC analysis. The measurement of airborne isocyantates presents inherent challenges and difficulties in both sampling and analysis based on their chemical and physical properties. Worker exposure to airborne isocyanates may be present in either the gas or particle phase, or both. To improve the performance of the reagent-coated glass fiber filters, field extraction of the filter has been recommended. Previous studies have identified a significant difference in the amount of MDI recovered of these two commonly used methods. This research was designed to

evaluate the accuracy of extraction, or desorption, of the filter in the field and laboratory following a liquid and aerosol spiking technique.

In Chapter 2, an innovative and novel isocyanate aerosol generating system (IAGS) to deliver known amounts of isocyante to test filters was evaluated. The IAGS was designed to emit a wide range of isocyaante aerosols to effectively model typical exposure scenarios encountered in polyurethane spray applications. Initial validation tests using water were completed to ensure the IAGS met design values and expectations. For example, delivery of isocyanates was accomplished by interfacing a KD Scientific 200 Two-Syringe Infusion Pump with an EZ-STARTER airbrush with an atomizing nozzle from PAASCHE. The reported accuracy and reproducibility of the KD Scientific 200 Two-Syringe Infusion Pump were ±<1% and ±<0.1%, respectively. Dispensed flow rates of 0.193 and 0.380 ml $min.^{-1}$ were validated with an experimental value of less than 1% error emitted from the IAGS. A subsequent pilot study, consisting of five samples, was conducted using an MDI working solution corresponding to 1.3 μg ml^{-1} of toluene dispensed at a flow rate of 0.193 ml $min.^{-1}$. This pilot study was a proof of concept and not an evaluation of the GFF method. With a standard deviation of 18 nanograms (ng) and a coefficient of variation of 8%, the IAGS was concluded to exceed expectations of consistent delivery of aerosolized MDI in order to evaluate the GFF method. MDI working solutions were prepared with toluene. Therefore, toluene was examined for potential interference with isocyaante deriviatization by 1,2-PP. Toluene was determined not to interfere with the reaction between MDI and 1, 2-PP based on the reported amounts of MDI following a series of dilutions with an increasing volume of toluene. Additionally, particle size distribution of the IAGS was characterized using a Grimm Dust Monitor Model 1.108. Data were approximately log-normally distributed and count median-, and mass median diameters were calculated for each

MDI working solution. The number and mass of particles cm^{-3} was normalized to the size range collected in each channel. An interval-normalized number and mass frequency plot were constructed to summarize the distribution. Approximately 95% of the aerosol mass concentration was associated with particles greater than 2 μm while 95% of the aerosol number concentration was associated with particles less 2 μm. The majority of the number concentration (75%) was contained between 0.35 and 0.725 μm. Particles greater than 3.5 μm contained 75% of the mass during the process. Some interesting effects of particle size and MDI concentration were observed in this study. Notably, the mass median diameter decreased with increasing mass concentrations of isocyanate. Since 2 μm is the recommended upper limit for using GFFs, ideally, the MMD would have been closer to 1μm to evaluate accuracy of sampling airborne MDI and TDI. However, the IAGS has provided a basis to model future experiments that spray load filters with known amounts of isocyanate. Overall, this project demonstrated that an isocyanate aerosol generating system could be developed for the purpose of spray-loading GFFs with known amounts of analyte to evaluate accuracy.

In Chapter 3, accuracy of the field (FD) and laboratory desorption (LD) methods was evaluated using a liquid spike and the IAGS from Chapter 2 to treat filters with previously underivatized technical and pure grade MDI. A range of working solutions was prepared to evaluate the FD and LD methods at small amounts of MDI. Additionally, using specific iterations of the flow rate of the syringe pump and a sampling pump, filter desorption methods were evaluated at, below, and above the OSHA PEL. A total of 191 MDI (i.e., technical and pure grade) samples were collected using FD and LD methods. Quantitative determinations of MDI from spray loading (n=105) and pipette loading (n=86) were reported in units of mass. Theoretical diisocyanate mass was calculated from the concentration of the working solution,

flow rate of the syringe pump, and total sampling time. Data were log-transformed to make inferences using a ratio of observed over theoretical, or percent recovery, to evaluate the accuracy and consistency of each diisocyanate field sampling method. Using the general linear model, a statistically significant three-way interaction was detected in the application of both technical and pure grade MDI, specifcially between loading mechanism, desorption methods, and loading concentration (p-value <0.05). This three-way interaction was not unexpected. Analytical results at each concentration were conjectured to vary with desorption method and loading mechanism. These results are consistent with previous work conducted in field experiments that demonstrated that filters desorbed in the field consistently produced higher amounts of MDI. Additionally, pipette loading of free MDI onto filter was anticipated to play a pivotal role in quantitative determinations of MDI as compared to spray loading. Generally, pipette and spray loading an FD and LD filter with a solution of underivatized MDI (i.e., technical and pure grade) yielded a significantly low percent recovery. For a fixed loading, statistically significant differences were detected between FD and LD (at specific loading concentrations) when either grade of MDI was sprayed onto a filter, but not when it was liquid spiked. Consistent with other studies, FD filters consistently yielded a higher percent recovery of MDI than LD filters. The observed statistical significant difference between FD and LD results related to airborne MDI were practically relevant. Underestimations of MDI in both FD and LD filters were attributed to reactions with water vapor or other hydroxyl radicals that may be present and simultaneously collected onto the filter. However, the loss of MDI was minimized in FD filters since the extracting solvent dissolved both the derivatizing reagent and any un-reacted isocyanate, allowing the two to combine in solution and form a stable urea-derivative. The LD filters, instead, were not desorbed for at least a few days considering shipping time. MDI

aerosols larger than 2 μm may have derivatized only a portion of the aerosol while the un-reacted portion was further exposed to humid air trapped inside the cassette after replacing the top cover and plugs. Using the mixed procedure in the SAS System, simple difference LSMEANs were analyzed between FD filters treated with technical and pure grade MDI, as well as LD filters. Compared to the technical grade MDI results, pure grade MDI was even further underestimated by both FD and LD filters whether they were pipette or spray loaded. The presence of other compounds in the technical grade may have partially shielded (since technical grade was still underestimated) the MDI from other reactants, facilitated dispersion of the MDI, or enhanced the analytical results.

The study in Chapter 4 evaluated the suitability of immediate desorption of filters treated with aerosolized pure grade TDI, using the IAGS, as compared to desorption at the analytical laboratory. Using the same study design as Chapter 3, five TDI working solutions were prepared with toluene to determine if concentration linearly predicted the percent recovery of TDI. A total of 50 TDI samples were collected using FD and LD methods. Data were log-transformed to make inferences using a ratio of observed over theoretical. Data were balanced indicating that the factors in this model were orthogonal. Using the general linear model, the omnibus test of the model showed a significant F-value of 5.26 (p-value <0.0001), the main effect of desorption alone was not found to predict percent recovery with an F-value of 1.26. The F-value associated with concentration was 9.34 (p-value < 0.0001). Concentration was linearly related to percent recovery after accounting for desorption. Significant two-way interactions between desorption and concentration was not observed. Generally, analytical results from each loading concentration of TDI did not vary with desorption method. Differences between FD and LD sampling methods were not significant following sample collection of atomized working

solutions 2-5. However, a significant difference was observed between FD and LD percent recovery of working solution 1, which emphasizes the importance of immediate desorption for a specific concentration of TDI composed of a particular fraction of gas and aerosol phases. FD and LD filters treated with atomized TDI demonstrated a statistically significant underestimation of recovered TDI compared to the theoretical amount across all working solutions. The profile of atomized TDI percent recovery was similar to the pure grade MDI results presented in Chapter 3. Loss of isocyanate to competitive reactions with water vapor most likely accounts for the low percent recovery observed in both this study and the MDI study. While volatilization of TDI may exclusively account for the loss of isocyanate (i.e., to the inside walls of the cassette), MDI has a much lower vapor pressure than TDI, and was not anticipated to volatilize during that study. As water vapor from ambient air was drawn onto the filter by the sampling pump, either hydrolysis of the isocyanate to its respective diamine occurred, or a polymeric urea was formed. As the concentration of TDI increased, the particle size distribution increased as well, causing agglomeration and less contact with the reagent-coated filter.

Taken together, the three studies demonstrate that while immediate desorption of isocyanate laden filters minimizes loss of the analyte, yielding higher amounts, field desorption significantly underestimates true amounts of isocyanate. Furthermore, it appears that accuracy of these sampling methods is not only governed by timing of desorption, the presence of other reactants, and the physical state, including size, of the isocyanate, but also the composition of the formula or product being applied. Finally, the studies provide evidence that continued research on current methods, or the development of novel methods able to efficiently derivatize an isocyanate, forming a reaction product that is detected readily and accurately, are essential to protecting workers from inadvertent exposures.

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DEDICATION

To my family, Lisa, Will, Mom, Dad, Sarah, Meghan and Jacob, my foundation and inspiration.

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°C	Degrees Celsius
cfm	Cubic feet per minute
cm	Centimeter
cm^2	Square centimeter
cm^3	Cubic centimeter
°F	Degrees Fahrenheit
L/min	Liters per minute
mL	Milliliter
ppm	Parts per million
psi	Pounds per square inch
µm	Micrometer
µL	Microliter
m	Meter
m^2	Square meter
m^3	Cubic meter
g	Gram
mg	Milligram
µg	Microgram
ng	Nanogram
mm	millimeter
mmHg	Millimeters of mercury
ppb	Parts per billion

LIST OF SYMBOLS

ρ_p	Symbol for density of particle (kilogram/m^3)
$D^3_{p,i}$	Midpoint aerodynamic diameter
d_a	Aerodynamic diameter
dM_i	Particle mass concentration
dN_i	Number concentration
D_p	Particle diameter
H_a	Alternative hypothesis
H_o	Null hypothesis
n	Number of samples
P_m	Measured pressure
P_o	Normal atmospheric pressure (25°C)
R	Gas constant (8.31 J/K·mol)
S_r	Saturation ratio
T_a	Ambient temperature
T_d	Temperature at droplet surface
T_m=	Measured temperature
T_o	Normal atmospheric temperature (760 torr.)
χ	Dynamic shape factor

CHAPTER 1—INTRODUCTION, GOALS, AND BACKGROUND

Introduction

Isocyanates are highly reactive chemicals that are used extensively as synthons in the manufacturing of polyurethanes (Karol 1986; Wisnewski, Hettick, and Siegel 2011; Wisnewski and Jones 2010; Wisnewski and Liu 2013; Wisnewski et al. 2013; Wisnewski et al. 2011; Melin et al. 2001; Nordqvist et al. 2001). Characterized by the presence of an NCO functional group attached to an aliphatic or aromatic parent compound, isocyanates are low molecular weight compounds. Isocyanates may be categorized as monoisocyanates (one NCO), diisocyanate monomers (two NCOs), or polyisocyanates (multiple NCOs) depending on the number of functional groups present (Streicher et al. 2000; Bello et al. 2004).

The diisocyanate monomers have gained widespread commercial and consumer interest due to a diverse number of end-use applications, such as flexible polyurethane foams (PUF), adhesives, sealants, elastomers, coating materials, and binders (Raulf-Heimsoth and Baur 1998). The presence of two, free NCO groups promotes polyaddition-polymerization through heteroatom cross linking of the isocyanate group and a monomer possessing a reactive hydrogen atom (e.g., -OH, -COOH, -NH2) (Malten and Zielhuis 1964). These monomers are typically polyfunctional alcohols, known as polyols (e.g., polyether polyol), which promotes a concerted, nucleophilic addition across the N=C bond of the isocyanate (Raspoet et al. 1998).

Methylene bisphenyl isocyanate (MDI) and toluene diisocyanate (TDI) are two of the most commercially relevant monomer products accounting for over 90% of the diisocyanate market (Raulf-Heimsoth and Baur 1998; Allport 2003). Domestic production capacity has been growing at a rate of 2.1% per year through 2004 and the trend is likely to continue at a high level as new markets and applications continue to develop (Allport 2003; Program 2009). As a result,

the number of workers at risk for over-exposures to MDI and TDI will also increase (Pronk et al. 2007). According to a National Occupational Exposure Survey, approximately 280,000 US workers are potentially exposed to MDI and TDI (Health. 2006).

The reactivity of MDI and TDI that contributes to their technical value in the polyurethane industry is expected to play a key role in toxicity (Ott et al. 2007). Inhalation is the main route of exposure that can elicit serious adverse health effects, which include: respiratory tract irritation, immune sensitization and asthma, respiratory sensitization, restrictive pulmonary disease, ataxia, hypersensitivity pneumonitis, reactive airway dysfunction syndrome (RADS), emphysema (Registry 2008). Isocyanates represent the major cause of occupational asthma induced by low molecular-weight chemicals (Allport 2003; Raulf-Heimsoth and Baur 1998; Pronk et al. 2007). The prevalence rate of sensitization and asthma in the exposed workforces has been reported as high as 20-30%. (Woskie et al. 2004)

The Occupational Safety and Health Administration (OSHA) has promulgated a set of ceiling Permissible Exposure Limits (PELs) of 20 parts per billion (ppb) for MDI and TDI monomers only (Administration). The American Conference of Governmental Industrial Hygienists (ACGIH) has published Threshold Limit Values (TLVs) based on a conventional 8-hour day and a 40-hour workweek for MDI and TDI, including a short-term exposure limit (STEL) 15-minute time weighted average (TWA) for TDI (Hygienists 2012) The National Institute for Occupational Safety and Health (NIOSH) has both a ceiling and a full-shift (10-hour) TWA recommended exposure limit (REL) for MDI (Health. 2010). The NIOSH recognizes TDI as an occupational carcinogen and advises that TDI exposures be reduced to the lowest feasible concentration (Health. 2010). Although not enforceable, NIOSH's REL and

ACGIH's TLV are more conservative occupational exposure limits and offer greater protection to workers.

Accurate sampling can be challenging and problematic because isocyanates may exist in the air as a vapor, an aerosol with a wide range of particle sizes, a coating on a dust particle, or as a partially reacted isocyanate-containing intermediate formed during POLYURETHANE production (Booth et al. 2009; Streicher et al. 2000). Airborne concentrations are influenced by several main factors, such as vapor pressures, and whether the isocyanate containing products are heated, sprayed, or poured. Workplace processes also play a key role in respiratory exposures, such as open or closed manufacturing processes (Streicher et al. 2000; Booth et al. 2009). In addition, thermal degradation of polyurethane materials, which can occur in a variety of settings that generate heat (e.g., welding, grinding, and cutting), can result in various vapor and particulate combustion products (Rom and Markowitz 2007).

The commonly used sampling methods that are the focus of this study are the OSHA Sampling Method 47 for MDI and 42 for TDI, and the Wisconsin Occupational Health Laboratory (WOHL) MDI/TDI Sampling Method LC 48. The OSHA Sampling Methods are based on laboratory desorption (LD) of the filter; both the cassette and filter are mailed to an analytical laboratory where extraction occurs in a solution of 90% acetonitrile and 10% dimethyl sulfoxide (90/10 ACN/DMSO) (Occupational Safety and Health Administration, 2008). On the contrary, the WOHL Sampling Method dictates that both monomer samples are field desorbed (FD) (*immediately* after sampling) in a collection vial containing two milliliters of 90/10 ACN/DMSO and then sent for analysis (Laboratory). While variants of sampling methods for MDI and TDI exist, companies have greater incentive to use the OSHA methods in order to

comply with current regulation. However, OSHA and WOHL methods differ in their post-sampling procedures, which may prove to be a methodological limitation or weakness.

Goals of dissertation research

In 2007, we conducted a side-by-side comparison of the OSHA sampling method 47 and the WOHL Sampling Method in an MDI-contaminated work environment (Schaeffer et al. 2013). Statistically significant differences were observed in airborne MDI sample concentrations; however, since this was a field study, accuracy of the sampling methods was not determined. Therefore, an overexposure to MDI may occur yet go unnoticed due to the sampling methodology.

We hypothesize that the WOHL sampling method is more accurate in estimating both MDI and TDI monomeric exposures due to the timing of filter desorption.

The significance of this study will allow agencies and occupational health practitioners to choose the most appropriate field-sampling method that best estimates worker exposures to MDI and TDI. As a result, appropriate and effective control measures may be implemented that mitigate the exposure to a protective metric.

Specific Aims

1. Develop and evaluate an isocyanate aerosol generating system to analyze the accuracy of glass fiber filter samplers used to monitor worker exposure to isocyanates. Previous evaluation of these methods was completed using a liquid- and vapor spiking technique to deliver pre-derivatized methylene bisphenyl isocyanate and toluene diisocyanate. Using the IAGS, isocyanate aerosols were used to evaluate the sampling methods. This study tested one main hypothesis: isocyanates can be accurately and consistently delivered in the aerosol phase at particle sizes representative of polyurethane spray applications. the methods were evaluated at

fundamental concentrations consistent with being above and below the Occupational Safety and Health Administration ceiling Permissible Exposure Limit.

2. Determine the accuracy of field and laboratory desorption techniques using a liquid spiking technique and the isocyanate aerosol generating system to deliver underivatized methylene bisphenyl isocyanate to test filters. A range of technical and pure grade working solutions of methylene bisphenyl isocyanate was prepared to evaluate the FD and LD methods at small amounts of MDI. Additionally, using specific iterations of the flow rate of the syringe pump and a sampling pump, filter desorption methods were evaluated at, below, and above the OSHA PEL. Based on our preliminary data, we know that one or both of the methods either overestimates or underestimates worker exposure to MDI. This study tested three hypotheses: 1) field desorption will yield higher amounts of methylene bisphenyl isocyanate that are more accurate than laboratory desorption; 2) liquid spiking filters will yield higher amounts of MDI compared to the IAGS based on enhanced derviatization kinetics provided by a solution environment; and 3) results from collecting pure grade methylene bisphenyl isocyanate samples will yield higher amounts of the analyte than technical grade methylene bisphenyl isocyanate due to the absence of other compounds.

3: Evaluate the suitability of immediate desorption of filters treated with laboratory generated aerosols of pure grade TDI, as compared to desorption at the analytical laboratory. A range of pure grade working solutions of toluene diisocyanate was prepared to evaluate the FD and LD methods at small amounts of TDI. Additionally, using specific iterations of the flow rate of the syringe pump and a sampling pump, filter desorption methods were evaluated at, below, and above the OSHA PEL. This study tested one hypothesis: collection of aerosolized TDI is

improved by immediate, or field, desorption rather than desorption at an analytical laboratory, which may take at least two days.

Background and significance

Major uses of MDI and TDI

Isocyanates were first synthesized in 1848 as esters of isocyanic acid (e.g.,methyl isocyanate) by the double decomposition of a dialkyl sulfate with potassium cyanate (Ozaki 1972; Ulrich 1996; Information. 2010). The chemistry of isocyanates was studied for almost a century before diisocyanates were recognized as model compounds capable of undergoing polymerization reactions to produce high-molecular products (Ulrich 1996). Polyurethanes (PUR) were invented by Bayer in 1937, and are the predominant end-use product of MDI and TDI (Cummings and Booth 2002).

Many PUR applications, such as coatings, adhesive, sealants, elastomers, and fibers evolved from Bayer's pioneered work, including a large demand within the flexible polyurethane industry (Cummings and Booth 2002; Kim 2008). Over 2.1 billion pounds of polyurethanes are produced and used in the US annually (Krone et al. 2003). Polyurethanes are the key constituent in cushioning material found in automobile seating furniture, bedding, and carpet pads. Polyurethanes are also utilized as semi-rigid and rigid foams (Kim 2008). These engineered materials can be tailored by the type and shape-memory polymers, which can recover original shapes, are designed with different phases of hard and soft segments that are diisocyanate-dependent, which may form varying degrees of physical cross-linking through polar interactions, hydrogen bonding, and crystallization (Zhang et al. 2010). Rigid polyurethanes exhibit a closed cell structure with superior mechanical properties (e.g., high compression strength, high strength-to-weight ratio, and low moisture permeability) and thermal stability (e.g., low thermal

conductivity) for use as insulation materials for construction and industrial applications, such as refrigerators, freezers, piping, tanks, and ship building (Kim 2008; Kang 2010).

Costs and high reactivity of isocyanates account for the commercial success of MDI and TDI, while the versatility of derived polymers ensures continued growth in a diverse commercial and industrial market. The major applications of TDI and MDI include flexible polyurethane foams (e.g., automotive seating); cast elastomers and coatings (e.g., sealants for construction industries and shoe soles); and rigid foams (e.g.,, thermal insulation of buildings). Both the type of isocyanate and polyol control the rigidity and flexibility of polyurethanes (see chemistry section).

In order to generate the characteristic branching and cross-linking observed in polyurethane addition polymers, alcohols with a hydroxy functionality of 2 (or greater) are used (Allport, 2003). While fully cured polyurethanes do not off gas and are completely stable at room temperatures, thermal degradation and mechanical operations may release traces of monomeric diisocyanate. Thermal lability of polyurethanes is directly associated with chemical structure of the alcohol. Phenol initiated diisocyanate polymerization results in a urethane product that is more easily reversed than its aliphatic counterpart, which has allowed for the development of customized polyurethane enamels.

Different polyurethane morphologies are tailored for function-specific applications by interchanging the use of MDI and TDI due to their symmetric and asymmetric structures, respectively. TDI derived polyurethanes are used extensively in the flexible slabstock foam industry (e.g.,, furniture and bedding), while MDI is suitable for preparing segmented polyurethane elastomers and rigid PUF foams (Booth et al. 2009; Cummings and Booth 2002). In fact according to the Alliance for the Polyurethanes Industry U.S. End-use Market surveys for

MDI and TDI, elastomers and reaction injection molds accounted for over half of the total percentage of MDI usage while approximately 70% of the total tonnage of TDI manufactured in the year 2000 was used in flexible slabstock foam.

General chemistry of MDI and TDI manufacturing:

While reaction paths are relatively simple, proprietary technology results in a variety of commercially available MDI and TDI formulations. These products exist in different chemical and physical forms to meet the needs for specific applications and emerging technologies, as illustrated in Figure 1-1(Allport 2003).

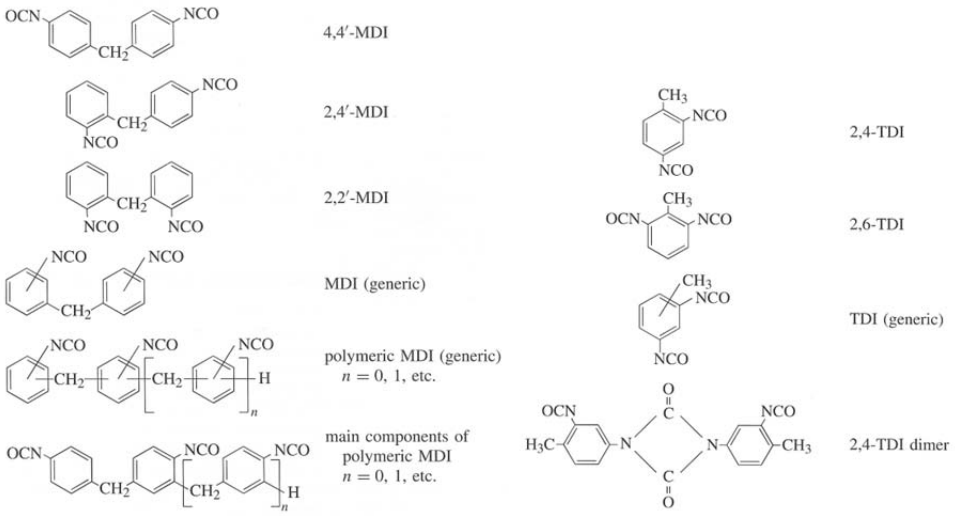

FIGURE 1-1. Chemical structures of MDI and TDI species (Allport 2003).

There are numerous methods for the preparation of isocyanates, most notably the phosgenation of an amine or its salt, despite the known toxicity of phosgene (Ozaki 1972; Cayli 2008; Saunders and Slocombe 1948). Concerns of accidental release of phosgene gas from plants resulted in salient efforts to develop nonphosgene processes. Double decomposition reactions, and Curtius, Hoffman, and Lossen rearrangements were a few to emerge (Saunders and Slocombe 1948). However, these reactions are rarely performed because of high chemical costs

and inherent danger with the required use of heat sensitive azides (Ulrich 1996). Introduction of noble metal (e.g., palladium, platinum, rhodium, and selenium) catalysts permits the utilization of carbon monoxide instead of phosgene (Bennet 1967). Briefly, a solution of nitro compound (e.g., dinitrotoluene) can be converted to an isocyanate in one step via reductive carbonylation reaction in the presence of carbon monoxide and a metal catalyst. Estimations of 18% and 83% in capital cost reduction and improvement in profit, respectively, were reported for a plant producing 100 million pounds per year (Ozaki 1972). However, technical problems surrounding the recovery and recycling of the catalyst from the homogenous residue proved to be too difficult and therefore not economically viable as originally thought.

Alternative methods have evolved, especially with considerations for low environmental impact. For example, room temperature ionic liquids have shown promise as an effective "green" solvent capable of mediating clean carbonylation reactions without any additional solvents and catalysts (Sima 2002). With unique chemical and physical properties, these solvents are achieving promising yields. Additionally, bio-based isocyanates were recently synthesized in two steps in 60-70% yields (Cayli 2008). The first step involved allylic bromination of soybean oil. Subsequently, a substitution reaction using AgNCO produced isocyanate-containing soybean oil. Polyurethanes were also synthesized in this study using castor oil, which portrayed low mechanical strengths, high elongations, and high swelling ratios appropriate for application in PUF. Castor oil has 2.7 hydroxyl groups per triglyceride that are evenly distributed, which allows for a uniformly crosslinked structure in the resulting polyurethane (Pfister, Xia, and Larock 2011). Vegetable oil is also emerging as a suitable substitute for polymer production. However, the fatty acid composition of vegetable oil does not contain naturally occurring hydroxyl groups like castor oil, but the carbon-carbon double bonds

and ester functionality permits hydroxyl group incorporation (Pfister, Xia, and Larock 2011). Soybean oil is readily used as it contains 4.6 double bonds per trigyceride capable of hydroxylation (Pfister, Xia, and Larock 2011).

Despite these cost-effective, eco-friendly breakthroughs, phosgenation of amines remains most important in large-scale, commercial production due to superior yields of a wide range of aliphatic and aromatic isocyanates from regioselective reactions, and technologies that now prevent release of phosgene (Cayli 2008; Ozaki 1972; Sima 2002; Ulrich 1996). Enclosed plants are capable of producing MDI and TDI in continuous processes, especially those plants with on-site manufacturing of petrochemical precursors (Allport 2003). Under pressure, aromatic diamines are reacted with excess phosgene in chlorobenzene under pressure (Ulrich 1996) Other solvents suitable for phosgenating aromatic diamines are benzene, toluene, xylenes; as well as polar solvents, such as ethyl acetate, dioxane, nitrobenzene or dimethylsulfone. Briefly, the key steps in manufacturing MDI and TDI are as follows (Figure 1-2):

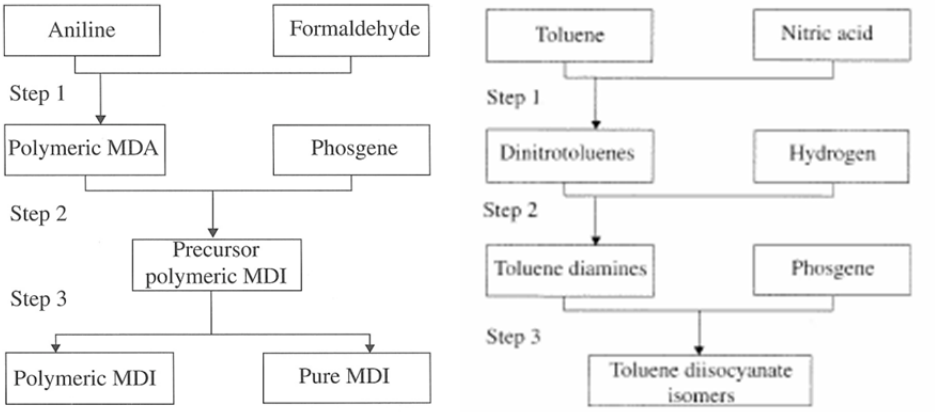

FIGURE 1-2. Key steps in manufacturing MDI and TDI (Allport 2003).

MDI Manufacturing

The principal reaction product in MDI manufacturing is known as polymeric MDI, which is an undistilled mixture containing monomeric (4, 4' MDI), and oligomeric isocyanates (Twitchett 1974). Oligomeric isocyanates are relatively low molecular weight polyisocyanates that contain 15 monomeric units or less, and a functionality of 3 or higher (Streicher et al. 2000; Allport 2003). However, due to the high reactivity of isocyanates, side reactions may occur. Consequently, the mean functionality of the commercial product is 2.8 –NCO groups (Twitchett 1974). Simultaneous production of monomeric and oligomeric isocyanates promotes the incorporation of by-products into polyisocyanates, which lends to quantitative yields (Ulrich 1996). Pure MDI, the difunctional species from which polyisocyanates and polyurethanes are derived, consists of at least 95% of the 4,4'-MDI isomer, which is achieved through distillation (e.g., continuous thin film or climbing film vacuum) (Streicher et al. 2000; Ulrich 1996).

Initially, MDI precursors, aniline and aqueous formaldehyde, are mixed in agitated reactors containing less than stoichometric amount of hydrochloric acid (HCl), or equimolecular proportions of HCl and aniline for pure MDI production. (Ulrich 1996). Condensation of aniline and aqueous formaldehyde is exothermic (-11 kcal mol^{-1} aniline reacted) and must be cooled to ensure prevention of undesirable side-reactions (Twitchett 1974). Intermediate products, typically secondary amines, are then gradually heated to 100°C over a two to four hour period. This second stage is known as isomerization, which converts these precursors into primary amines. While a mixture of methylene diphenyl diamine and higher oligomers are expected, the percent composition of reaction products will depend on the ratio of starting materials and reaction conditions (Allport 2003). This mixture is also known as polymeric methylene diphenyl diamine.

Phosgenation of aromatic amino constituents with increasing functionality present in polymeric methylene diphenyl diamine is readily converted into aromatic isocyanates (Figure 1-3) (Allport 2003; Ulrich 1996; Saunders and Slocombe 1948; Twitchett 1974). The reaction is initiated at room temperature, and in a step gradient fashion is elevated to temperatures of 180°C (Ulrich 1996). The reaction is usually carried out in a solvent medium, typically monochlorobenzene, because it is inert to phosgene, hydrogen chloride, and the isocyanate prepared (Twitchett 1974).

FIGURE 1-3. Basic reaction scheme between aromatic amine and phosgene to yield an aromatic isocyanate (Allport 2003).

Hydrogen chloride and excess phosgene by-products contribute to the reaction mix, and are continuously vented, distilled, and recycled, leaving behind a polymeric MDI reaction product (Allport 2003). Recovered hydrogen chloride is reused in the aniline/formaldehyde condensation reaction, sold, or oxidized to chlorine. Phosgene can be prepared from the oxidized chlorine (Ulrich 1996).

The polymeric MDI reaction product is a mixture of monomeric di- and multi-ring isomers, including pure MDI (4, 4' isomer) (Figure 1-4) (Ulrich 1996; Allport 2003). Using crystallization methods or vacuum distillation (either thin film or climbing film), pure 4,4' MDI is removed, leaving behind a polymeric residue, consisting of 2,2'- and 2,4'-isomers of MDI, as well as oligomeric isocyanates with a functionality of 3 or higher (Allport 2003). The rich fraction of 4, 4' MDI is purified further to remove chlorine-containing impurities and residual isomers to produce technically pure MDI (Twitchett 1974).

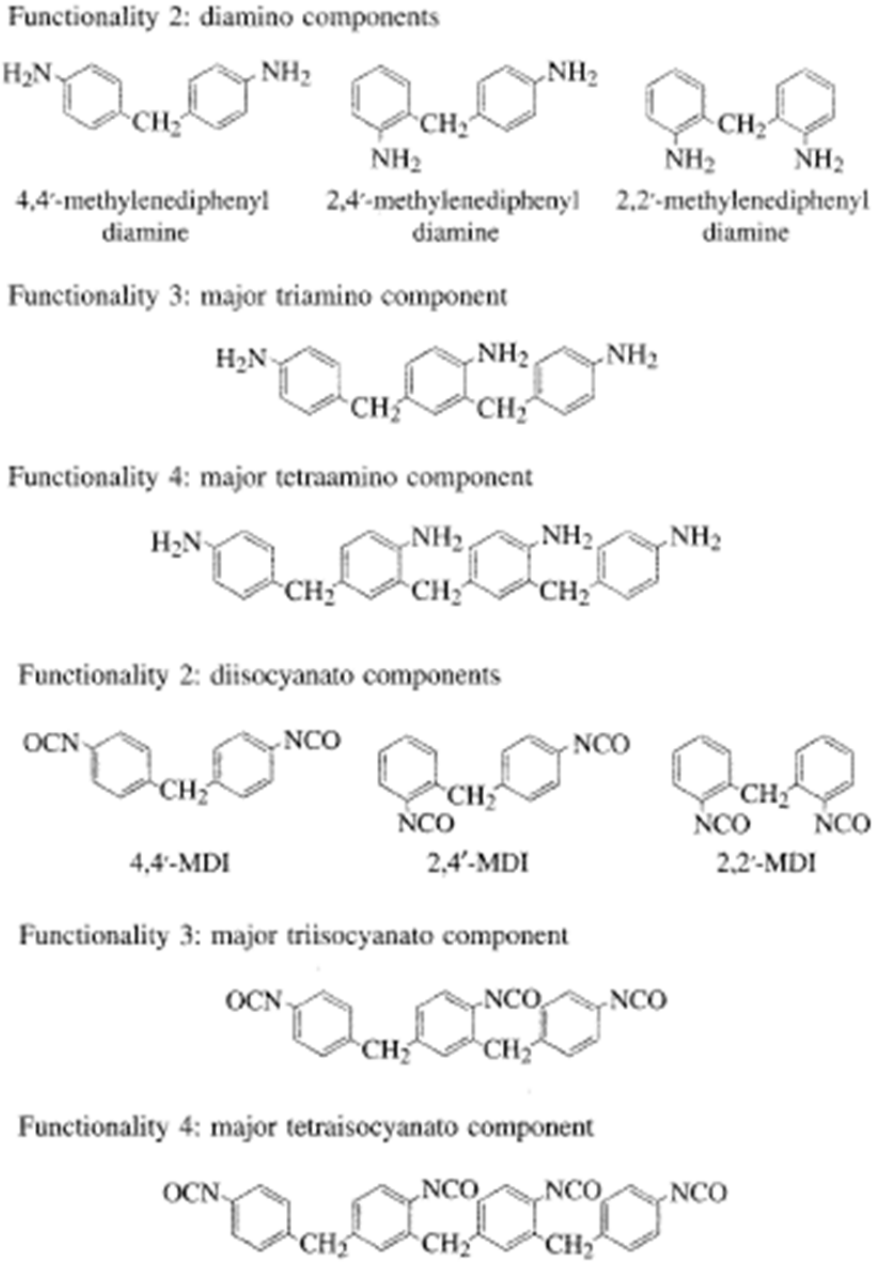

FIGURE 1-4. Mixture of methylene diphenyl diamine and MDI species of different functionality that are present during manufacturing (Allport 2003).

TDI Manufacturing

The mechanism of the phosgenation reaction in MDI manufacturing is also fundamental in the production of TDI. However, the precursors are quite different. The chemistry involves treating toluene with a mixture of nitric and sulphuric acids to give dinitrotoluene (Twitchett

1974). Other processes use high strength nitric acid, which eliminates handling of spent sulfuric acid (Ulrich 1996). Dinitrotoluene is removed from the acid bath, neutralized and washed (Twitchett 1974). Dinitration of toluene is a two-step process that affords a mixture of the 2,4'- and 2,6'-isomers (Figure 1-5) (Allport 2003).

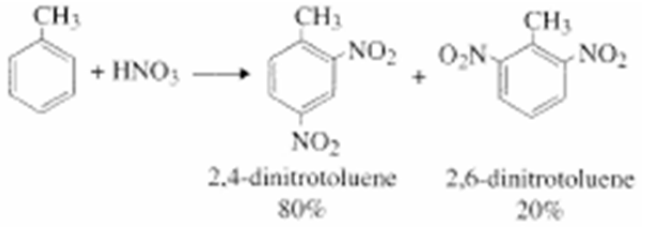

FIGURE 1-5. Dintration of toluene (Allport 2003).

Initially, mononitrotoluene isomers, specifically 2-, and 4-nitrotoluene are formed during the first stage. Upon further nitration, 2,4'- and 2,6'-dintirotoluene isomers are produced as an 80:20 product (Ulrich 1996). Exclusive production of 2,4 dinitrotoluene or 65:35 mixuture of 2.4- and 2,6 dinitrotoluene may also be achieved by isolation of either 4-nitrotoluene or 2-nitrotoluene, respectively (Figure 1-6) (Allport 2003).

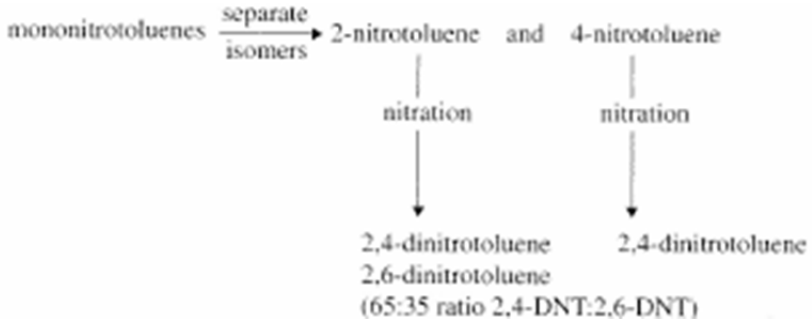

FIGURE 1-6. Isomeric mixtures of dinitrotoluene, precursors of TDI (Allport 2003).

In the past, the mixture of isomeric dinitrotoluenes was reduced to toluene diamines using iron and aqueous acid, which also prevented unwanted ortho-diamines, including 2,3-, and 2,5-toluene diamine isomers, through involatile iron coordination complexes (Twitchett 1974; Allport 2003). These impurities may increase turbidity as a result of spontaneous

cyclopolymerization. Currently, catalytic hydrogenation is now performed at 120°C under pressure in an alcohol solvent (e.g., methanol) in the presence of Raney nickel and excess hydrogen to generate higher yields of desired meta-diamines (Twitchett 1974; Ulrich 1996). One caveat to hydrogenation of dintrotoluenes is the formation of nitrocreosols during dinitration of toluene (Twitchett 1974). These by-products act as catalytic poisons, and therefore must be minimized by specific reaction conditions.

Separation of toluene diamine isomers, specifically the desired meta-derivatives, must be completed prior to phosgenation (Ulrich 1996). Crystallization methods are too costly for large-scale purification (Twitchett 1974). Falling film evaporator provides best practice for distilling toluene diamine into a 96% phosgenating-ready isomeric mixture (Ulrich 1996). Remaining ortho-compounds can be removed using either fractional distillation or additives (e.g., boric acid) that react preferentially with ortho-amino groups (Ulrich 1996; Twitchett 1974).

Originally, toluene diamine was phosgenated in single stage processes, or batchwise, in a stirred reactor containing halogenated aromatic solvent and phosgene (Twitchett 1974). Continuous phosgenation has since replaced batchwise production for greater efficiency and better TDI yields. toluene diamine and excess phosgene in chlorobenzene are added in parallel to a series of stirred vessels, the first a well-agitated reactor (Twitchett 1974; Ulrich 1996). Conditions of temperature and pressure and reactor design varies among manufactures, but generally phosgenation is conducted at about 100°C and 155 lb/in^2 gage pressure at a molar ratio of 10:1 $COCl_2$/toluene diamine(Ulrich 1996; Twitchett 1974).

Continuous flow of the reaction mixture from first reactor to the last allows crude isocyanate to be concentrated and distilled to approximately 99% pure product with the removal of residual solvent, hydrochloric acid by-products and excess phosgene (Allport 2003; Ulrich

1996; Twitchett 1974). Vented hydrogen chloride and distilled solvent are re-used (Allport 2003).

Chemical and physical properties

MDI and TDI are contained in many commercial products under a variety of convenient names and formal nomenclature for which the manufacturers' material safety data sheets should be consulted for detailed information on product identity and properties. For instance, TDI is a colorless to pale yellow liquid with a sharp acrid odor and a boiling point of 251°C (Twitchett 1974; Hygienists 2004)

The Chemical Abstracts Service (CAS)™ Registry and the International Union of Pure and Applied Chemistry (IUPAC) are two universal systems of nomenclature (Allport, 2003). The CAS Registry uses a system of unique numbering to identify both pure chemicals (including isomer mixtures) and reaction products of either defined or undefined structures. For example, the CAS number and preferred name for 4,4'-MDI is 101-68-8 and benzene, 1,1'-methylenebis[4-isocyanatobenzene, 1-isocyanato-2-[(4-isocyanatophenyl)methyl-. IUPAC is based on a formal system of nomenclature that describes only defined structures; the IUPAC system does not address mixtures of chemicals (such as the 80:20 TDI). 2,4-TDI according to the IUPAC system is 2,4-diisocyanato-1-methylbenzene.

General nomenclature (e.g.,, mono-, di-, and poly-) of isocyanates describes the number of NCO functional groups in the molecule that are attached to a specific moiety, namely an aliphatic or aromatic structure. MDI and TDI are aromatic diisocyanates that include industrially important congeners distinguished by spatial substitution of two NCO groups, which react orders of magnitude faster than aliphatic diisocyanates (Ulrich, 1996).

The term monomeric TDI usually refers to the 80:20 ratio of 2,4'- and/or 2,6'-TDI isomers, which represents over 95% of industrial usage (Hygienists 2004). See chemical and physical properties below (Hygienists 2004):

Molecular weight: 174.15
Specific gravity: 1.22 at 25°C
Freezing point: 11.5° to 13.5°C
Boiling point: 251°C
Vapor pressure: 0.02 torr at 25°C
Flash point: 132°C, open cup
Solubility: soluble in diethyl ether, acetone, and other organic solvents
Reactivity: contact with water, acids, bases, and amines may cause uncontrollable polymerization and rapid evolution of heat
Decomposition product: in dilute solutions, formation of free diaminotoluene is possible
Convervsion factors at 25°C and 760 torr: 1ppm= 7.12mg/m^3; 1mg/m^3= 0.14ppm

Conversely, technically pure MDI is a low melting solid, usually presenting as white to light-yellow, odorless flakes (Twitchett 1974; Hygienists 2001). See chemical and physical properties below (Hygienists 2001):

Molecular weight: 250.26
Specific gravity: 1.197 at 70°C
Melting point: 37.2°C
Boiling point 194° to 199°C
Vapor pressure: 0.00014 torr at 25°C; 0.001 torr at 40°C; 1torr at 170°C; and 10 torr at 205°C
Solubility: soluble in octane, benzene, and kerosene; 0.2% soluble in water (g/100g H_2O at 20°C)

However, MDI distribution may be in the form of liquid derivatives. Polymeric and modified MDI exist (depending on the formulation) in a brown to translucent brown liquid, with varying isocyanate content and viscosity (Allport 2003). Modified MDI (or TDI) is converted from monomeric species for easier handling and increased versatility in final polymer properties. Modified MDI or TDI products are also termed variants or prepolymers.

Workers are potentially exposed to unreacted monomers, prepolymers, oligomers, and/or polyisocyanate species depending on the application of the isocyanate. These isocyanate-related terms are defined as follows (Streicher et al. 2000):

> **Monomer**: a difunctional starting material capable of deriving polyurethanes (and polyisocyanates)
>
> **Prepolymer**: a less volatile species possessing free isocyanate groups prepared by reacting a polyol in an excess of diisocyanate
>
> **Oligomer**: a low molecular weight polyisocyanate comprised of (approximately) 15 monomeric units
>
> **Polyisocyanates**: a homopolymer possessing free isocyanate functional groups, generated by linking monomeric units of MDI or TDI

In general, exposure potential for MDI and TDI is interdependent on their vapor pressure and use. Accordingly, potential for vapor exposure to MDI is low, as it does not readily volatilize at ambient temperatures. However, if mechanically aerosolized or heated in the work environment, vapors and particulates with wide range of particle sizes will pose as an inhalation hazard (Booth et al. 2009). On the other hand, vapor pressure of TDI is orders of magnitude higher; therefore exposures to both TDI vapor and aerosols are possible depending on expected application of the product (Cummings and Booth 2002; Laboratory)

Chemistry

The unique feature of diisocyanates is their disposition to react readily and undergo either oligomerization, homopolymerization, or heteropolymerization; each reaction forming dimers, polyisocyanates, and polyurethanes (PURs), respectively (Henneken, Vogel, and Karst 2007). Heteropolymerization is a polyaddition reaction that involves compounds containing active hydrogens (X—H), e.g., polyols, which proceed after moderate heating and without catalysts

(Saunders and Slocombe 1948). However, addition of other compounds such as tertiary amines accelerate the reaction time.

The molecular orbital theory predicts the greatest charge density on the oxygen atom, imparting the highest net positive charge on the carbon, which accounts for the high reactivity and wide spectrum of reactions of isocyanates. The major and minor resonance structures are shown in Figure 1-7. (Arnold 1957)

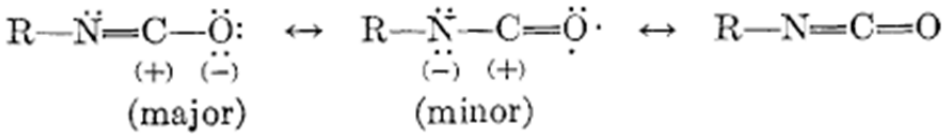

FIGURE 1-7. Resonance structures of isocyanates (Arnold 1957).

Any active hydrogen compound can react with the isocyanate groups to produce addition compounds by a nucleophilic attack upon the electrophilic carbon (Ashida 2007; Arnold 1957). Reactivity of the X—H and NCO moiety increases as nucleophilicity and electrophilicity increases, respectively (Arnold 1957). For example, when stronger electron-withdrawing groups are attached to isocyanate entity, a greater positive charge will be placed on the carbon.

Polyols are compounds with multiple hydroxy functionality that serve as nucleophilic agents that react instantly with diisocyanates in a one-step polymerization process (Ulrich 1996). For example, ethylene glycol is a molecule with two hydroxy groups, also known as a diol. The hydroxy functionality of at least two allows for addition across the N=C double bonds to form repeat units of NHCOO groups characteristic of PUR products (Ashida 2007). The chemical backbone of polyols varies significantly (e.g.,, polyester, polyether, polyester-polyether hybrids, or hydroxyl-containing vegetable oil) according to dimension specifications needed of the polymer (Ulrich 1996; Ashida 2007).

Blending of polyols of different functionality also facilitates tailoring for a specific application (Ulrich 1996). However, polyether polyols (both aliphatic and aromatic) are the main building blocks of PURs. The structure (or spatial positioning) of the hydroxy group is obtained from initiators, such as alcohols or amines that can vary in functionality. For instance, flexible foams may use initiators of functionality two, e.g., dipropylene glycol, where as rigid foams may use sucrose, which has a functionality of six. Polyether polyols of functionality 2-3 are high molecular weight and usually linear.

Conversely, higher functional polyether polyols (3-7) are low molecular weight compounds that are highly branched polymers. Polypropylene oxide homopolymers or block copolymers typically provide the backbone for polyether polyols due to their formation of terminal hydroxyl groups in the primary position by ethylene oxide capping. Chain extenders are used to either finish polymerization of isocyanate-terminated prepolymers, or in the productioin of thermoplastic polyurethane elastomers production.

Reactions

Homopolymerization reactions

The homopolymerization of either MDI or TDI is an important reaction in isocyanate technology to reduce vapor hazards associated with lower molecular weight diisocyanates (Streicher et al. 2000). Direct linkage of up to 10 monomeric units of isocyanates through condensation of non-hindered isocyanato groups results in a polyisocyanate that contains multiple free NCO groups that can further react to produce a polymer of greater complexity (Bello et al. 2007; Ulrich 1996; Bello et al. 2004; Streicher et al. 2000). Linear polymers of TDI and MDI are coupled through selective reactions and electron transfers using catalysts (e.g.,

sodium cyanide or samarium iodide) in a polar aprotic solvent to minimize side reactions (Ulrich 1996).

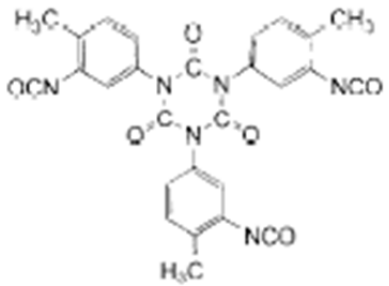

FIGURE 1-8. TDI polyisocyanate with multiple functional groups free to react (Streicher et al. 2000).

Copolymerization reactions

Polyisocyanate configuration (Figure 1-8) may also arise from anionic polymerization that initiates an MDI (or TDI) reaction with either vinyl monomers (e.g.,, styrene or methyl methacrylate), or a copolymer (e.g.,, di- or polyfunctional alcohols or amine) (Ulrich 1996; Streicher et al. 2000). Organolithium reagents, such as n-butyllithium, catalyze initiation reactions that are several orders of magnitude faster than propagation (R.A. Godfrey 1969). Therefore, block polymers are prepared that contain pendant reactive isocyanate groups, which in turn may be crosslinked in subsequent reactions with water, diols, and diamines.

Cyclopolymerization reactions

Cyclopolymerization is a type of self-reaction of aromatic diisocyanates across the functional group that is capable of producing oligomeric dimers (uretidinediones) and trimers (isocyanurates) (Figure 1-9) (Ulrich 1996). Exceptions are aromatic diisocyanates with NCO groups in the ortho position, which homopolymerize and cause turbidity in commercial TDI (Kober 1969).

Dimers are insoluble contaminants that are avoided in polymerization reactions as they adversely affect the physical properties of the polyurethane (Allport 2003). Dimerization is an exothermic, [2+2] cycloaddition reaction that usually requires phosphines or pyridine catalysis (Allport 2003). Ortho-substitutions to the isocyanate functional group are more prone to homopolymerizations than dimerizations, suggesting MDI is more likely to form dimers as compared to TDI (Karol 1986). Pure MDI in the crystalline state will dimerize slowly at room temperature because of proper alignment of the reacting groups within this matrix (Roxy B. Wilson 1983). Noncatalyzed dimerization of TDI has not been reported (Ulrich 1996). Temperatures above 55°C are also suitable conditions for dimerization. Therefore, rapid cooling (in distillation or thermal treatment) and cold-room storage of both liquid and solid forms are essential for minimizing heat-induced dimerization (Ulrich 1996; Allport 2003). Additionally, heating at high temperatures (i.e., 200° C), dimers will dissociate back into monomers (Karol 1986). Such reversibility is a putative source of exposure with attendant health effects encountered by firemen in certain situations.

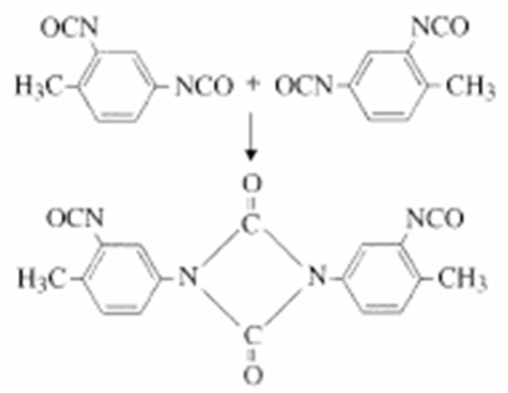

FIGURE 1-9. Self reaction of 2,4 TDI to form dimer (Allport 2003).

Trimerization of aromatic diisocyanates (Figure 1-10) is a preferential reaction with the formation of highly stable cyclic isocyanurates containing three reactive groups capable of chain

branching and cross-linking (Allport 2003). These reactive derivatives are highly applicable as rigid foams with inherent fire resistant properties, conferred from the ring structure (Ulrich 1996).

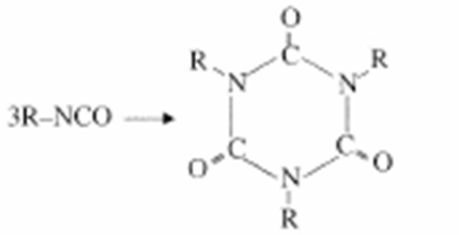

FIGURE 1-10. Trimerization of TDI (Allport 2003).

Reactions with hydroxylated compounds

The polarized, azomethine-like linkage of the aromatic structures of MDI and TDI promotes the formation of a carbamic ester, or urethane, when reacted with an alcohol or phenol (Saunders and Slocombe 1948; Ozaki 1972; Allport 2003). The reaction scheme illustrated in Figure 1-11 is the basis of PUR manufacturing.

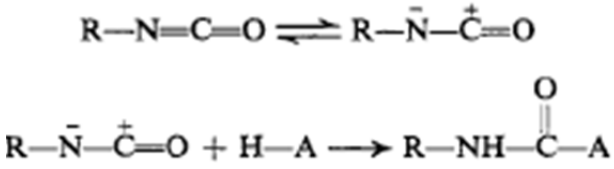

FIGURE 1-11. Nucleophilic addition to the polarized azomethine linkage of the isocyanate functional group (Ozaki 1972).

Polyols, specifically diols, are likely alcohol candidates for PUR production with molecular weights ranging from 250 to 8000 (Ulrich 1996). A simple representation of a polyaddition reaction between a difunctional isocyanate and diol is shown in Figure 1-12.

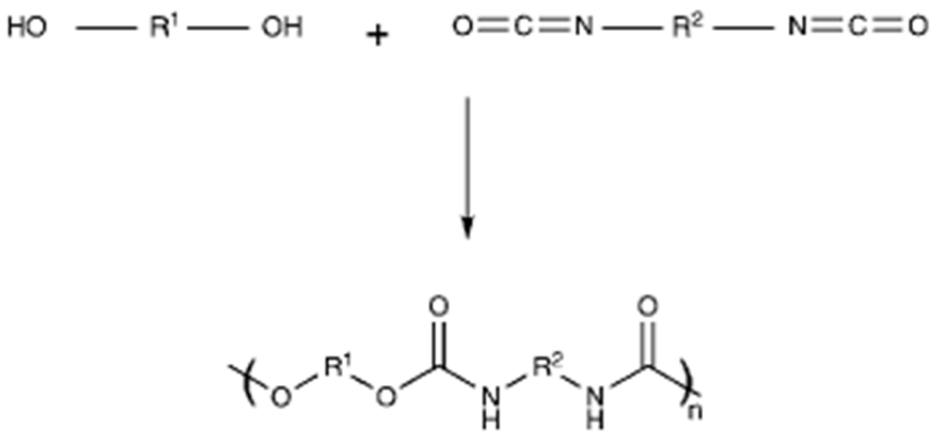

FIGURE 1-12. Basic reaction scheme between a diisocyanate and diol to form PUR (Pfister, Xia, and Larock 2011).

Using a kinetic model (Figure 1-13), a permutation of oligourethanes may be created that can react with each other, or with the monomeric precursors to produce a polyurethane via step polymerization (Krol 1995):

$$A + B \xrightarrow{k_1} A_1B_1$$
$$A_1B_1 + A \xrightarrow{k_2} A_2B_1$$
$$A_1B_1 + B \xrightarrow{k_3} A_1B_2$$
$$A_1B_1 + A_1B_1 \xrightarrow{k_4} A_2B_2$$

FIGURE 1-13. Representation of step polymerization reaction mechanism (Krol 1995).

While relative reactivity of MDI or TDI with hydroxyl compounds can be predicted using addition reaction schemes, reaction rates are sterically influenced; therefore, these factors must be considered (Saunders and Slocombe 1948). Hindered reactions are the cause of both the isocyanate and alcohol structures. These culprits have contributed to an array of reaction rates. For instance, primary NCO groups react faster than secondary and tertiary NCO groups in aliphatic isocyanates (Ulrich 1996). However, MDI and TDI are more reactive than aliphatic isocyanates due to the electrophilic effect of their aromatic substituents, but ortho substitution on these aromatic diisocyanates lowers their reactivity (Bello et al. 2004; Ulrich 1996).

Polyoxypropylene ether polyols are comprised of mainly secondary hydroxyl groups (Ashida 2007). While multiple hydroxyl functional groups are available for these organic reactions, the relative reactivity of primary alcohols to secondary is 3:1. Investigators have concluded that the reaction rates, using unreacted isoycanate as an index for measurement, of primary alcohols greatly surpass their secondary and tertiary counterparts (Gemeinhardt 1960). However, secondary hydroxyl-containing polyoxyproplyene triols and diols have shown capabilities in one-shot polyurethane foam using appropriate metal catalysts (Gemeinhardt 1960). Briefly, the metal coordination complex formation is the likely reaction mechanism facilitating use of such alcohols. Catalytic activity, measured in gelation time of the mixture, showed a few metals to greatly increase the speed of the reaction, notably bismuth and lead.

Early experiments elucidated a first-reaction rate when using equimolar concentrations of alcohols and isocyanates in aprotic solvents (Gaunt 1949). These solvents promoted second-order rate constants through a decrease in alcohol concentration from self-association reactions. In 1995, Krol proposed a general kinetic scheme by quantifying rate constants for two irreversible reactions for gradual chain growth polyaddition process for the synthesis of linear chain polyurethanes based on the reaction between difunctional isocyanates and diols/polyols (e.g., polyethers) (Krol 1995). This study showed that the structure of the reactants had the greatest effect on the rate of reaction compared to other influencing variables, such as temperature, catalyst, and reactant size.

Raspoet and Nguyen provided mechanistic evidence for the alcoholysis and hydrolysis reactions of isocyanates from both experimental and theoretical results (Raspoet et al. 1998, 1998). Using 2-propanol and cyclohexanol in both low and high concentrations, the alcoholysis

of isocyanates followed a multimolecular mechanism involving concerted nucleophilic addition of either two or three molecules of alcohol across the N=C bond (Raspoet et al. 1998).

Theoretical results, using ab initio methods to model the hydration mechanism by water and water clusters, suggest a second-order dependence on water (Raspoet et al. 1998). Briefly, two water molecules in the form of a dimer, nearly linear in structure, were revealed to play a pivotal role in the preferential reaction mechanism, as the activation energy for one water molecule only was unrealistic, in terms of Fukui functions, when considering structures of the reactants, transition states, and products. Putatively, a third water molecule in the water chain is ideal as it lowers the energy barrier by bridging a gap so correct orientation is achieved for a concerted nucleophilic addition across the N=C bond, just like in the above alcoholysis reaction scheme.

The first patent of a flexible polyurethane foam preparation was awarded in 1942, which was based on the reaction between TDI and water, which produces CO_2 as foaming agent to produce PUR chains (Ashida 2007). This reaction can be highly exothermic with serious risk of combustion, as well as emanation of unreacted monomers by evaporation or CO_2 conveyance to the atmosphere(Malten and Zielhuis 1964).

Additionally, MDI and TDI reactions with water are important in the context of environmental impact. The fate of MDI and TDI in the aquatic environment can be summed up in two sequential steps: hydrolysis and urea formation (Allport 2003). The hydrolysis of MDI and TDI yields an unstable carbamic acid that readily decarboxylates to an amine (most likely MDA or TDA) and carbon dioxide. The amine will react with excess isocyanate to form a urea. The inherently low solubilities and slow rates of dissolution of MDI and TDI limit their dispersion in water. These factors help protect the environment from vigorous, exothermic

reactions. MDI and TDI have higher densities relative to water, and therefore will sink. Globules will form according to their hydrophobic nature, minimizing surface area that can interface with water. The rate of urea (specifically polyurea) production is dependent on slow diffusion of water into the reaction zone, forming a "crust" from the outside inwards—which minimizes availability of MDI and TDI and precludes diffusion of MDA and TDA. While polyureas are solid, insoluble, and stable in the environment to both chemical and biological attack, entrapped carbon dioxide may facilitate globule mobility by causing temporary flotation.

Carboxylic acid reactions

Isocyanate –carboxylic acid reactions yield an unstable mixture of anhydrides (Allport 2003). Subsequent decomposition is spontaneous or heat induced, which leads to two different products depending on the disposition of the starting materials. For instance, weak aromatic and aliphatic carboxylic acids will generate both a carboxylic acid anhydride and a substituted urea at elevated temperatures (Arnold 1957). When strong acids, e.g., formic acid, are used instead, spontaneous decomposition results in amide and CO_2 production.

Reactions with –NH groups Amine, carbamate, and urea reactions

Primary and secondary amines and amides are also compounds containing an active hydrogen that is capable of nucleophilic addition to an iscyanate (Ozaki 1972). Basicity or nucleophilicity of the –NH bond is used as an index of reactivity to indicate production of substituted ureas or acyl ureas by either amines or amides, respectively (Saunders and Slocombe 1948). Steric factors are also a governing principal in reaction rates of these –NH compounds with isocyanate species. Relatively, the reaction of amines with isocyanates is much faster than alcohols. However, the reaction between aromatic diisocyanates and aliphatic diamines has not achieved the same importance as the reaction with difunctional alcohols due to the formation of

allophanates and biurets that occurs at higher temperatures from unreacted isocyanates further reacting with these generated ureas (Ulrich 1996; Allport 2003). Therefore, hydroxyl group containing monomers are often reacted simultaneously with amino group containing monomers to generate poly (urethane ureas), which allows polyurethane modifications through crosslinking and chain extending (Ulrich 1996; Allport 2003).

Mercaptan and thiophenol reactions

The reaction of isocyanates with mercaptans and thiophenols generates thiocarbamates (thiourethanes) under suitable conditions (Allport 2003). The thiol group is less reactive than its oxygen analog when present in nonionizing conditions (Arnold 1957). However, when polarizing solvents are used or basic compounds added to the solution, the reaction rate is much faster (Friedrich 1959). This, in turn, may favor isocyanate reactions with sulfur containing compound, and promote preferential reaction even in the presence of alcohols.

Biological molecule reactions

The reaction mechanisms described above are vital to understanding the biophysics of exposure to MDI and TDI, as well as the identification of key cellular and molecular players to determine pathogenesis of disease, most notably occupational asthma. Upon uptake of MDI or TDI, biologically relevant physicochemical forms of isocyanates by which these species elicit their effects is difficult to define due to rapid reactions with proteins and water (Wisnewski and Jones 2010). In a structure-activity relationship model, this inherent reactivity of diisocyanates and their ability to bind to macromolecules is a key feature that distinguishes them as sensitizing chemicals as opposed to non-sensitizing (Karol, Macina, and Cunningham 2001). MDI and TDI can self-react to yield homopolymers, dimers, or trimers (Karol 1986). These polymers are capable of reacting with proteins at single or multiple sites, resulting in cross-linking of different

polypeptide chains by intra- or intermolecular linkages (Raulf-Heimsoth and Baur 1998). Proteins and other biological macromolecules (e.g., nucleic acids, carbohydrates, and lipids) contain active hydrogen compounds that are reactive to these isocyanates (Allport 2003). For example, protein sites contain amino acid side chains comprised of hydroxyl, carboxyl, thiol and aliphatic amino groups that are often free to react.

Carboxylic acids are also key players in metabolic intermediates that play a vital role in the citric acid cycle and de novo syntheis of essential biochemical compounds. Glutathione is a tripeptide featuring thiol groups, which is widely expressed in bodily fluids and tissues (Allport 2003). Such variety of reactive groups increases the potential for interactions and competitive reactions in the biological system, in turn posing salient challenges in defining primary events unequivocally associated with the health effects of isocyanate exposure.

Consideration of reaction selectivity as related to both the electrophilic center of MDI and TDI and nucleophilic centers of these endogenous functional groups offers insight into the likelihood of a particular reaction occurring (Coles 1984; Allport 2003). Chemical hardness and softness are terms used to describe the polarizability of the electrophilic and nucleophilic center of reactants (Coles 1984). While highly reactive, the polarized double bonds of diisocyanates are most likely considered a soft electrophile as compared to the alkyl carbonium ions, which has a higher positive charge density. Therefore, MDI and TDI predominantly react with soft nucleophiles, such as thiol groups of cysteinyl residues in proteins and glutathione, and primary carboxyl and amino groups in protein (e.g., lysine).

When the molecular orbitals of diisocyanates and biological molecules are mixed, they must pass through a high-energy transition state before generating a reaction product (Coles 1984). Similar molecular orbitals between reactants provide less of an energy barrier, therefore

affording reaction selectivity and rapidity. Thus, a reaction between diisocyanate and proteins and glutathione are more likely to occur than with harder nucleophiles, such as sites in ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). However, depending on microenviroment, biotransformation of diisocyanates may yield metabolites, for instance methylenedianiline (MDA), that are capable of forming DNA adducts, as illustrated in figure in Figure 1-14 (see Toxicology section for additional information):

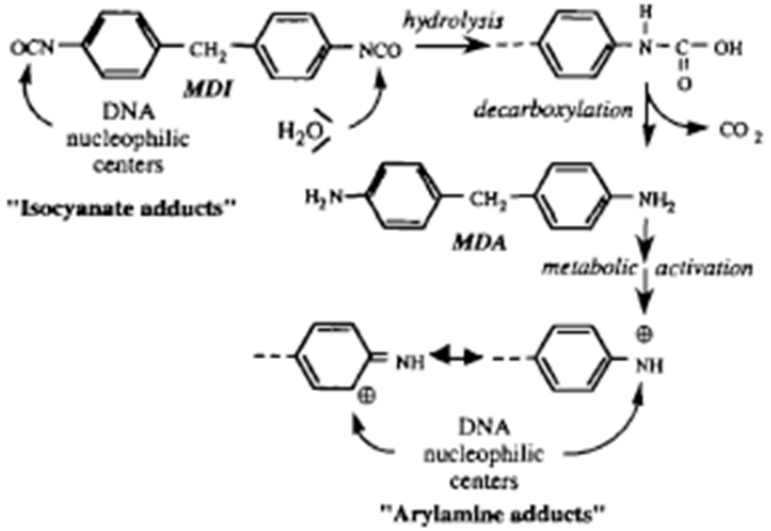

FIGURE 1-14. Biotransformation and bioactivation of MDI (Vock et al. 1996).

While many assumptions and hypotheses have been postulated for indirect and direct pathomechanisms and their putative etiology of induced diseases, the leading hypothesis is that the chemical acts as a hapten in vivo, which is recognized by the immune system subsequent to nucleophilic addition reactions with "self" proteins (Ye et al. 2006). Conjugates are most likely with airway and blood proteins, e.g., albumin, which is the most abundant blood plasma protein with carrier functions. All potential cascading effects upon isocyanate uptake are shown in Figure 1-15.

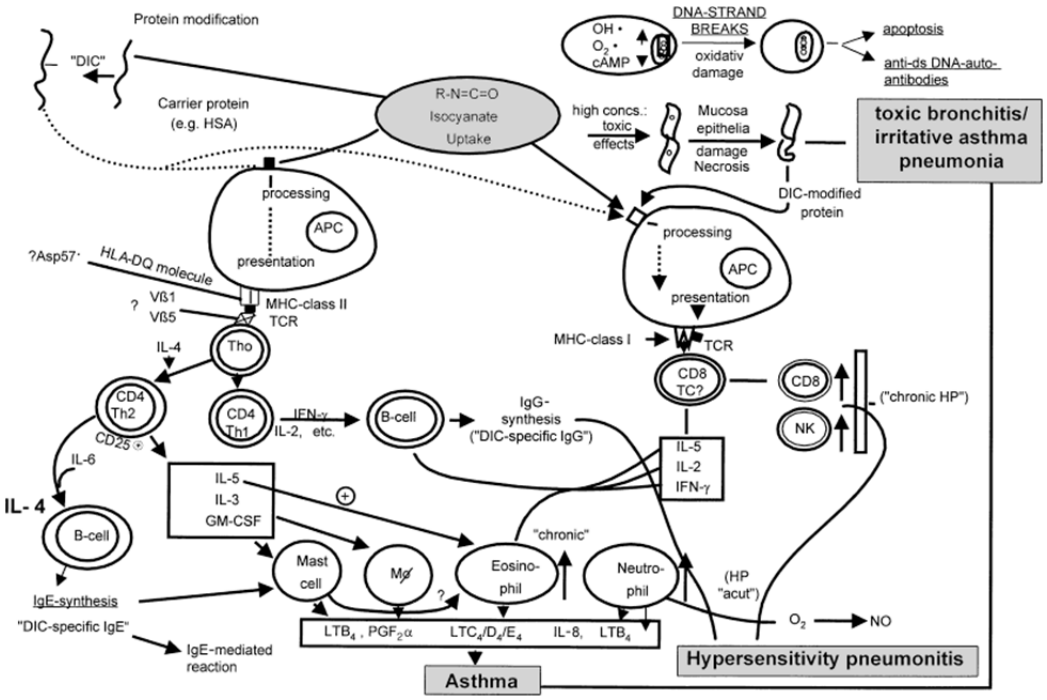

FIGURE 1-15. Pathomechanistic representation of isocyanate uptake and clinical endpoints (Raulf-Heimsoth and Baur 1998).

In the Lange et al. study using cell cultures of human bronchial epithelial cells, TDI vapor concentrations of 20 ppb and 100 ppb both showed colocalization of TDI with ciliary tubulins using confocal microscopy with double staining (Lange et al. 1999). Tubulin is an important globular protein of ciliary microtubules that converts ciliary motion to cellular function via the cytoskeleton. While this overlap may indicate altered signal transduction, these effects need further understanding, especially to determine if the colocalization was a result of co-occurrence or correlation of interaction.

Isocyanates are susceptible to nucleophilic attack by specific amino acids that yield a defined adduct products under physiological conditions, for example: the α-amino group of the *N*-terminal amino acids (e.g., valine and aspartic acid), the sulfhydryl group of cysteine, the hydroxyl groups of tyrosine and serine, the ε-amino group of lysine, and the imidazole ring of histidine (Kennedy and Brown 1992; Brown et al. 1987).

Brochoaveoloar lavage fluid attained from human subjects following diisocyanate exposure showed that the predominant conjugation of diisocyanates was albumin (Mapp et al. 2005). Wisnewski et al. in 2010 identified 14 MDI conjugation sites under physiological exposure conditions while characterizing structural determinants of the antigenic MDI-albumin conjugate using a variety of analytical methods (e.g., high-performance liquid chromatography and tandem mass spectrometry). Human albumin's evolutionary distinct amino acid sequence, specifically dilysine motifs, was ascertained to set this carrier protein apart from other proteins with strong primary sequence homology (Wisnewski, Liu, and Redlich 2010). Consequently, these lysine side chains, on the carboxyl side of another lysine, provide preferential targets by which diisocyanates can form conjugates.

Identification of these amino acid residues provides compelling mechanistic evidence of a likely reaction from a wide variety of available biological molecules with inference to the toxic effects of diisocyanate exposure. Additionally, lysine residues with biological functional activity are emerging as specific targets for covalent and noncovalent chemical binding of other low-molecular-weight chemicals attributed to immune-sensitization.

Toxicology

Exposure to isocyanates has been associated with neurological, dermal, respiratory, and immunological effects with clinical manifestations that include dermatitis, skin and respiratory tract irritation, immune sensitization and asthma, and hypersentitivity pneumonitis (Karol 1986; Bello et al. 2004). Other symptoms (e.g., hematologic and gastrointestinal) and fatalities have been reported in isolated cases (Booth et al. 2009; Karol 1986; Chester et al. 2005). Chemical carcinogenesis via genotoxic and nongentoxic mechanisms has also been postulated for MDI (Vock et al. 1996; Sepai et al. 1995).

Experimental toxicology with subcutaneous and intratracheal injections of TDI began as early as 1941 to determine systemic effects and physiological endpoints from exposure (Malten and Zielhuis 1964). When large quantities of isocyanate were applied to these animal models, a uniform pattern of prodromal signs and subsequent irritative and allergic effects were observed. Death was precipitated with 0.3ml injections of TDI, causing respiratory distress from protein coagulation. In 1956, a human subjects study to determine irritant and odor thresholds was conducted using TDI aerosol. Exposure time of one-minute to 0.13, 1.9, and 3.9 ppm demonstrated aromatic detection (without irritation), chemical conjunctivitis, and severe irritation in eyes and respiratory tract, respectively. In 1960, possible pathogenic mechanisms of isocyanate exposure were summarized as follows:

- Toxic damage specific to isocyanate reactivity
- Allergic response to mucosa protein debris
- Allergic response to bacterial colonizing in damaged mucosa
- Allergic response specific to isocyanates

Toxicity differences between MDI and TDI likely exist due to properties of the parent moiety attached to the NCO functional group, deposition site in the lungs, or attendant solvent exposures (Bello et al. 2004). Three key properties accounting for toxicity are electrophilicity, lipophilicity, and three-dimensional structures. The octanol-water partition coefficient of MDI and TDI were obtained using and high-performance liquid chromatography (HPLC) and reported as log K_{ow} of 4.5 and 3.4, respectively (Allport 2003). Therefore, MDI may penetrate biological barriers and reach biologically susceptible sites faster than isocyanates with lower lipid solubility (Pauluhn 2002). This concept was proven in a comparative analysis of MDI and hexamethyelene diisocyanate (HDI) to determine relative potency, using total protein and

angiotensin-converting enzyme as endpoints in bronchoaveolar lavage fluid to represent transient pulmonary irritation and perturbation of blood-air barrier (Figure 1-16) (Pauluhn 2002).

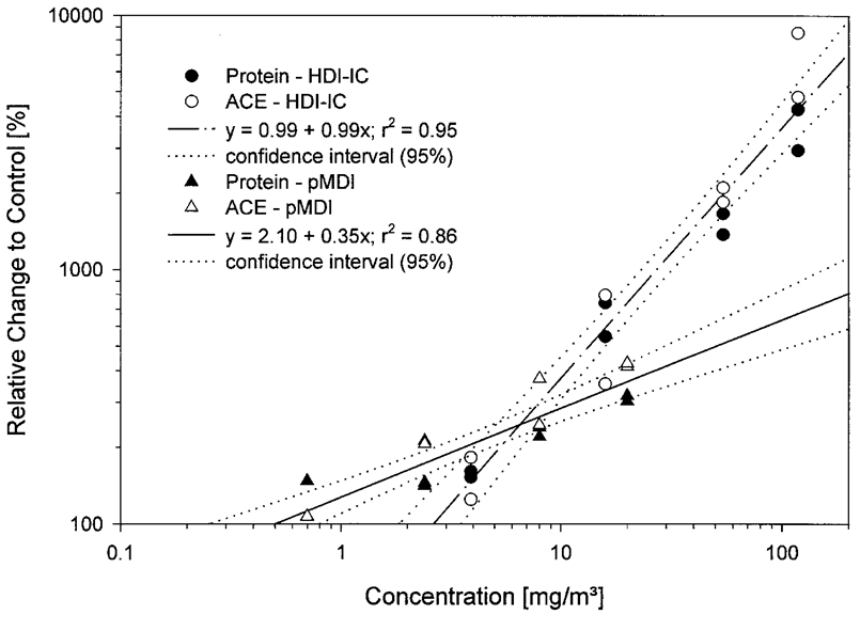

FIGURE 1-16. Graph representation of MDI and HDI potency (Pauluhn 2002).

Potency was determined to be directly proportional to MDI's lipophilic properties and aromatic-induced electrophilic effect due to different slopes rather than shifts in concentration effect curves (not shown). Using no-observed-adverse-effect-levels (NOAELs), toxicity differences of 17 mg NCO/m^3 and 160 mg NCO/m3 for MDI and HDI monomers, respectively, are almost an order of magnitude (Bello et al. 2004).

However, comparing these NOAELs is not entirely accurate as these levels represent different outcomes using specific exposure protocols. Exposure settings and uses of diisocyanates widely vary to draw any conclusions related to toxicity differences between monomers, specifically MDI, TDI and HDI. Two different studies using specific challenge protocols illustrated that MDI, TDI, and HDI all induce sensitization and asthmatic responses at similar doses of isocyanate (Malo, Ghezzo, and Elie 1999; Vandenplas et al. 1992).

Lange et al. conducted *in vitro* experiments on the effects of TDI vapor on human bronchial epithelial cells (Lange et al. 1999). At concentrations of 100 ppb or higher, cytotoxicity was evident by cell pyknosis and DNA fragementation. Toxicological responses associated with MDI and TDI exposures are dependent on a few variables, which include (Allport 2003):

- Route of exposure
- Duration of exposure (peak vs. average)
- Concentration of diisocyanate at target site
- Bioavailability of reactive biological molecules
- Prevailing biological pH
- Chemical (e.g., unreacted monomer) and physical form (vapor, aerosol, or liquid) of the diisocyanate

Wegman et. al. identified a dose-response relationship of TDI in workers producing PUR for mattresses and auto seat cushions (Wegman 1974). Participation included 111 first shift production workers that were grouped according to exposure categories, which were based on reproducible air samples at each job of the plant using Marcali method (see Method section). These exposure groups were then classified into three different groups based on less than, equal to, or greater than the TLV of 5 ppm. Using worker FEV_1 results from pulmonary function testing within each exposure group on a Monday following three days away from work showed a mean change that is significantly different from zero. Workers exposed to concentrations as low as 0.002 ppm showed acute loss of pulmonary function. Using a stepwise regression of independent variables collected using a questionnaire and change in FEV1 during work shift as dependent variable, the magnitude of exposure to TDI was the only significant variable. While a

dose-response relationship was illustrated in this population of workers exposed to low levels of TDI by acute loss of FEV1, chronic loss of pulmonary function is also expected at prolonged exposure levels of 0.002 ppm.

Reuzel et al. exposed rats to different test atmospheres of polymeric MDI (which contains 50% of monomeric MDI) aerosols in a series of single and repeated exposures (6 hours per day and 5 days per week) consistent with acute (4-hour), and subacute (2-week), and subchronic (13-week) time periods (Reuzel, Kuper, et al. 1994). A four-hour lethal concentration in 50% of the population (LC50) was produced at 490 mg/m^3 of respirable polymeric MDI. Mortality with a reduction in body weight and grayish wet lungs with pulmonary hemorrhages and nasal discharge resulted following single exposures in all concentrations tested (i.e. 384, 418, 500, 523 mg/m^3).

Concentrations in excess of 12 mg/m^3 also produced mortalities in subacute and subchronic studies with evidence of pulmonary toxicity at lower concentrations. The respiratory tract was designated as the target organ system of polymeric MDI inhalation. Concordantly, Reuzel et al. conducted a chronic inhalation toxicity and carcinogenicity study on exposure to polymeric MDI aerosols over 24 months (Reuzel, Arts, et al. 1994). Four groups of 60 Wistar rats were exposed to aerosol concentrations of 0, 0.2, 1.0, or 6 mg/m3 for 6 hours a day, 5 days per week. Localized effects were observed at 1.0 and 6.0 mg/m3 in the respiratory tract consistent with exposure to irritant aerosols. Basal cell hyperplasia of the nasal olfactory epithelium and accumulations of alveolar macrophages containing yellowish particulate material concomitant with fibrous tissue were evident. Eight pulmonary adenomas and one pulmonary adenocarcinoma were observed in the highest exposure group. With increased incidences of alveolar bronchiolization in this group, Reuzel et al. concluded that recurrent alveolar wall

damage by aforementioned macrophages explains the small incidence of these bronchoalveolar tumors.

Chronic exposure to insoluble particulates has long been investigated in inhalation studies involving rats using a wide range of materials. Tumorigenesis is the result of persistent inflammatory responses, including epithelial hypertrophy and/or hyperplasia and squamous metaplasia, to a lung load of particulates that exceeds normal clearance capacity either by overwhelming the alveolar macrophage mediated mechanism, or by induction of toxicity by biologically reactive particles (Hext 1994). At the molecular level, cytokines, an inflammatory mediator, is playing a putative role in the progression of the tumorigenic response. While mechanistic studies and elucidation of other key molecular players are still evolving, the rat model is perceived to be highly susceptible to the particulate induced pulmonary tumors. As of 2002, the current view on neoplastic mechanisms in polymeric MDI-induced pulmonary tumor involve a non-gentoxic epigenetic route directed by persistent irritation and epithelial hyperplasia (Kilgour et al. 2002).

Using a similar experimental design to Reuzel et al. in both inhalation studies, Kilgour et al. conducted acute and repeated sub-acute inhalation exposures to polymeric MDI in rats to evaluate early changes in lungs while assessing capacity for recovery following short-term exposures (Kilgour et al. 2002). Test atmospheres were generated in an exposure chamber consistent with respirable fractions of MDI only (mass median aerodynamic diameter ca. 1). While the artificial generation of MDI aerosols may not completely represent workplace conditions, results from this investigation are of toxicological interest that highlights an initial pattern of toxicity consistent with exposure to irritant aerosols. For instance, using histo-pathological and cellular proliferation techniques interfaced with electron microscopy, a large

influx of inflammatory mediators results from acute exposures to MDI were detected immediately after exposure with concomitant cellular damage marked by cellular exudate and debris in the lumen, and increased lung surfactant present 3-days post-exposure. As shown in Figure 1-17, biochemical, cellular, and pathological changes are evident.

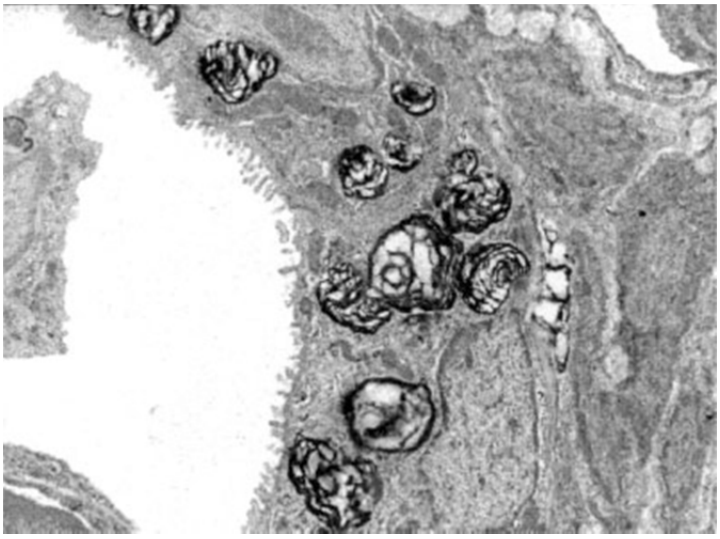

FIGURE 1-17. Increased number and size of lamellar bodies within type II alveolar cells after 3 days post-exposure to polymeric MDI atmospheres of 100mg/m^3 (Kilgour et al. 2002).

Other parameters measured were total cell count, including neutrophils and alveolar macrophages; in addition, enzyme activity, specifically lactate dehydrogenase and N-acetyl-β-D-glucosamindase in lavage fluid, which is suggestive of alveolar lumen insult. Alkaline phosphatase in the lung lining was also measured with notable elevated activity, which is attributed to type II cell toxicity or stimulation and increased pulmonary surfactant production. After a 10-day recovery period, all effects had recovered back to baseline.

Repeated exposures to less toxic concentrations of 1,4 or 10 mg/m^3 occurred over a 28-day period. Irritant effects were reproduced in these exposures, but less severe than those observed in acute exposures. Lung lavage exhibited "foamy" macrophages from the two highest

exposures along with increased phospholipid content. Histological results reflected bronchiolitis and cento-acinar thickening related to cellular proliferation in these regions. After a 30-day recovery period, all effects had recovered back to baseline.

With rapid recovery of effects in the lung in both the acute and sub-acute exposure and cessation of bronchiolar epithelial hypertophy/hyperplasia, which is a sentinel marker of neoplasia, fortifies the etiological hypothesis of these pulmonary tumors, which are subsequent to chronic irritant effects and insoluble polyureas formed by polymerization of polymeric MDI (see Hext above).

A series of studies have investigated the mutageneticity and genotoxicity of both MDI and TDI. In 1980, Anderson et al. performed a plate incorporation assay using histidine-requiring mutants of Salmonella typhimurium (Anderson 1980). Using test strains TA 98 and 100 various kinds of mutagens can be detected that cause frameshift and base pair substitution, respectively. With the presence of rat liver microsome fraction S-9 and NADPH-generating system, MDI and TDI were considered mutagenic to both T 98 and T100 following metabolic activation to their diamine analogues, specifically MDA and TDA. Results were classified as positive if the agar incorporated isocyanates produced a ratio of 2:1 of revertant colonies to the spontaneous number and if that number increased with dose.

While Anderson et al. suggested that aromatic diamines are the culprit responsible for mutagenic effects of diisocyanates, Marczynski et al. conducted *in vivo* MDI inhalation and *in vitro* TDI exposure studies to further determine genotoxic effects to evaluate risk in the occupational arena (Marczynski, Czuppon, Hoffarth, et al. 1992; Marczynski et al. 1993). Using a challenge chamber, a patient was continuously exposed to MDI atmospheres, ranging from 5-20 ppb for no more than 30 minutes (Marczynski, Czuppon, Hoffarth, et al. 1992). Isolated

white blood cell DNA was obtained pre- and post-inhalation and was analyzed by electrophoresis, anion-exchange chromatography, and melting behavior. Electrophoresis showed additional bands other than native genomic DNA fragments, which suggests cross-linking in DNA. Results from anion-exchange chromatography complemented these post-inhalation findings with an additional peak of white blood cell DNA that was step-wise eluted at 35% salt concentration as opposed to only one genomic DNA peak in the control at 78% salt.

When human blood was treated with TDI, DNA isolated from white blood cells showed single- and double-strand breaks *in vitro* (Marczynski, Czuppon, Marek, et al. 1992). Cross-linked TDI-treated DNA was evident after denaturation and renaturation. However, TDI-induced-DNA damage was not observed when purified DNA was treated with the isocyanate, suggesting that biotransformation to metabolites is a prerequisite for genotoxicity or mutagenicity. Additionally, the immediate effect of TDI on genomic DNA of sheep and rabbit white blood cells was investigated (Marczynski et al. 1993). While similar results were attained as in the *in vivo* inhalation exposure test, specifically chromatographic elution patterns in treated vs. control, chromosomal DNA from rabbit blood was further studied for isocyanate-induced apoptosis using the "agarose-plugs" method by pulsed-field gel electrophoresis with quantitative evaluation performed by laser densitometer. Prednisone, a known accelerator of apoptosis, was used as a positive control. Laser densitometer results indicate that TDI can induce degradation of mitochondrial DNA and large DNA fragments, which are the result of apoptosis, to small DNA fragments (Marczynski et al. 1993).

Vock et al. developed a ^{32}P-postlabeling method to determine if MDA-derived DNA adducts played a significant role in toxicity upon inhalation of MDI aerosol (Vock et al. 1996). Wistar rats were exposed 18 hours per day, 5 days per week for one year in whole body

chambers and subsequently euthanized for detection and quantification of DNA adducts. The lung did not exhibit MDA-DNA adduct, which is surprising given the aforementioned results of Reuzel and Hext. MDA-DNA adduct formation was detected in the olfactory epithelium with a supra-linear dose response curve. Trapping of MDI aerosols may occur in the ethmoid turbinate due to the tortuous architecture, consequently promoting bioactivation of MDI into MDA via hydrolysis and decarboxylation reactions in the mucous-laden upper respiratory tract. MDA, a known animal carcinogen, may also react with MDI to form polyureas, chemically inert particles, which also exert carcinogenic effects. In this case, MDI played a procarcinogen role, thereby extending is potency and contributing to genotoxic and nongenotoxic aspects of carcinogenesis.

Hemoglobin adducts were also investigated in the same animals using a similar exposure scheme (Sepai et al. 1995). Similar dose-response curves were observed using two different hemoglobin isolations and adduct quantitation techniques. These results are incongruent to Vock et al. as the presence of hemoglobin adducts suggest systemic bioavailability of MDA rather than localized interactions. Formation of lung DNA adducts may prove too difficult due to the:

- High reactivity of diisocyanates with cells lining epithelium of lung, and
- Long diffusion pathways in bronchiolar/alveolar region, and
- Proteins contain nucleophiles that are equivalently reactive (similar hardness), and
- Lifetime of erythrocytes are longer and without repair mechanisms

Therefore, isocyanate-protein conjugates are more likely to occur, and offer exposure artifacts that can be used to biomonitor exposure to MDI and assess a risk resulting from such exposure (Sepai et al. 1995). Additionally, since the molecular weight of diisocyanates is less than 1000 Da, theoretically MDI and TDI should act as haptens that can cause sensitization

through IgE or non-IgE mediated mechanisms (see Sensitization Section?) (Rom 1998; Yucesoy and Johnson 2011)

The International Agency for Research on Cancer has classified MDI under Group 3, which is not *classifiable as to its carcinogenicity to humans*. This classification is consistent with *inadequate evidence* for carcinogenicity in humans, and *limited evidence* in experimental animals exposed to a mixture containing both monomeric and polymeric MDI (Cancer 1999). However, TDI is classified as a reasonably anticipated human carcinogen (Group 2B) (Program 2009).

TDI is classified by the IARC as a Group 2B carcinogen due to the incidence of cancer in experimental animals upon gavage administration of commercial mixtures of 2,4- and 2, 6-TDI. A dose-related increase in subcutaneous fibromas and fibrosarcomas, pancreatic acinar- and islet-cell adenomas were observed in male rats. In female rats and mice, mammary gland fibroadenomas and hepatocellular adenomas were observed, respectively. On the contrary, no TDI-related tumors developed from inhalation exposures by mice and rats (Cancer 1986).

Industrial cohort studies and population-based case-control studies have been conducted to examine the potential association between occupational exposure to isocyanates and several types of cancer. Investigators monitored employees (both men and women) during at least a six-month employment period between 1958-1979 at 11 factories in England and Wales (Sorahan and Pope 1993) and 1958-1987 in nine Swedish factory (Hagmar, Welinder, and Mikoczy 1993). Both of these studies found no significant increase in mortality for cancers related to isocyanate exposure. Some cancers showed an increase in frequency among the female employees, but confounding variables (e.g.,, smoking or zero exposure) were attributed to the cause of increase incidence. Sorahan and Hagmar updated their studies with nine and 11 more years of follow up

data, respectively. In the updated Sorahan study, no positive trends were associated with isocyanate exposure and risks of lung cancer or non-malignant diseases of respiratory system (Sorahan 2003). Hagmar's study was unable to link isocyanate exposed employment with lung cancer risk, but suggested that occupational risk factors should not be excluded due to the limitations of retrospective studies and more in-depth exposure analyses are essential (Mikoczy et al. 2004).

Routes of exposure

Inhalation

Inhalation is the major route of occupational exposure to MDI and TDI, which results in significant physiological effects attributed to either a direct toxic or allergic response (Woolrich 1982). Depending on the physicochemical state of either diisocyanate, the extent of penetration and absorption will vary widely, imparting a range of symptoms from mild irritation to life-threatening effects (Allport 2003). Repeated exposures may result in long-lasting respiratory complications, most commonly occupational asthma. Additionally, PUR workplaces utilize other materials that are recognized pulmonary irritants (e.g., tertiary amine catalysts, solvents, and wood dust), which may accompany isocyanate inhalation exposures, posing as potential confounders when diagnosing diisocyanate asthma (see sensitization and immunology section). Large quantities of MDI and TDI are not absorbed unchanged through the respiratory tract due to immediate reactions with biomolecules such as glutathione, mucopolysaccaharides and proteins present in the moist environment (Allport 2003).

Animal inhalation studies with ^{14}C-labled MDI and TDI suggest that pulmonary absorption of these chemicals occurs in the upper airways, specifically epithelial and subepithelial layers from the nose down to the terminal bronchioles (Gledhill et al. 2005;

Kennedy et al. 1989). Gledhill et al. used ^{14}C-labled MDI to determine bioavailability in the rat model following inhalation. A systemic estimate of 25% of the received dose was ascertained based on detection of radioactive compounds in a multitude of tissues and carcass residual. The largest percentage of activity was associated with respiratory and GI tracts (Gledhill et al. 2005). Kennedy et al. proposed a linear rate of initial uptake into the blood following inhalation using ^{14}C-labled TDI ranging from 0.00005 to 0.146 ppm (Kennedy et al. 1989). A slight increase in uptake was also noted post-exposure. The linear rate was determined to be a function of isocyanate concentration, which also directly affects the development of hypersensitivity (Karol 1983).

Oral ingestion from nasopharyngeal deposition with subsequent mucocilliary clearance, and grooming of contaminated fur most likely account for the majority of the systemic availability of radiolabeled MDI (this is an important toxicological consideration when extrapolating animal data to occupational exposure since the internal dose may be higher than expected) (Gledhill et al. 2005). Additionally, 80% of the received dose was detected in feces, which would not be plausible had the exposure been exclusively from inhalation. Parent MDI was not detected in urine or bile, which demonstrates the reactivity of the compound. These last two statements suggest that direct absorption of isocyanates via inhalation is negligible while immediate reactions and formation of conjugates are highly anticipated biological fates.

Dermal

Dermal contact is an important route of isocyanate exposure in occupational settings causing either allergic or irritant contact dermatitis, including eczema and urticaria, which likely contributes to sensitization and asthma (Bello et al. 2007; Liu et al. 2009; Allport 2003). For instance, assuming 1% skin absorption of an MDI droplet containing 10 milligrams, an internal

dose that is 450 times greater than the current short-term UK occupational exposure limit of 15-min, 70μg NCO/m^3, which is based on total reactive isocyanate groups, regardless of origin (e.g., monomer or polyisocyanate) (Bello et al. 2004).

Skin exposures are likely to occur during spills, cleanup, and contact with contaminated equipment as a result of poor occupational hygiene (e.g., direct contact with unprotected skin) or failure of personal protective equipment (PPE), such as gloves (Bello et al. 2007). The role of skin exposure in isocyanate sensitization and asthma depends on a few structurally- and physicochemical-related properties, such as molecular mass, lipophilicity, and chemical reactivity, which may cause variation between MDI and TDI congeners. The condition of the biological barrier may facilitate skin absorption, as is the case in eczema and other sequelae, abraded regions (including shaving), and hand washing. Coexpsoures to solvents, additives, and excipients also used in PUR formulations may enhance dermal absorption of isoycanates, as well as break through of PPE—specifically gloves.

Numerous industrial accidents involving spill and splashes to the hands, face and whole body have been equivocally linked to the development of isocyanate sensitization (Karol 1986). Exploration of isocyanates as reactive haptens capable of producing respiratory tract hypersensitivity was initiated in a guinea pig model in 1981 (Karol et al. 1981). Using TDI concentrations ranging from 1-100% and applying a 50μl aliquot on the shaved region of the dorsal site, Karol et al. showed results indicative of both delayed-onset cutaneous and respiratory hypersensitivity. Contact sensitivity was apparent seven days post-exposure. Serologic analysis and bronchial provocation challenge were relied upon to associate dermal exposure with respiratory sensitivity two weeks post-exposure. TDI-specific antibodies showed a dose-response relationship between concentration of TDI used and titer of antibodies produced. Immediate

reflex increase in respiratory rate from exposure to low concentrations of TDI or aerosols of TDI-protein conjugates was confirmed in a percentage of animals (4 out of 12), using a criterion based on pre-determined background response in control (nonsensitized) guinea pigs.

Other studies applied MDI and TDI to the skin of albino rabbits, which elicited mild irritation marked by erythema and edema (Woolrich 1982). Desquamation and fissuring of the skin were associated with single TDI dosages that repaired by the 14th day of observation.

While increased awareness of potential risks of isocyanate skin exposure in the work environment is evident, a significant gap in knowledge remains with such exposures along with their interaction (e.g., synergistic) with respiratory exposure, especially low-level expsoures. Even with the advent of improved controls and less-volatile isocyanate substitution, more studies are needed to ensure safer work environments and worker health.

Qualitative and quantitative methodologies to assess skin exposure and relate its contribution to immunologic, respiratory and other outcomes have been proposed as part of the SPRAY (Survey of Painters and Repairers of Autobody by Yale) study, in collaboration with the University of Massachusetts Lowell (Liu et al. 2007; Bello et al. 2008; Liu et al. 2009). To date this cross-sectional epidemiologic study in autobody shops has:

- Revealed the utility of colorimetric indicators to document isocyanate surface migration and skin exposure, including under routine PPE (Liu et al. 2007), and
- Developed a quantitative wipe sample method based on NIOSH 5525 method (Bello et al. 2008), and
- Demonstrated that exposures to auto body shop workers is common during painting, mixing and paint-related tasks (Bello et al. 2008), and

- Provided a semiquantitative algorithm for task-based personal daily and weekly estimates of isocyanate skin exposures (Liu et al. 2009).

Ingestion

Animal experiments have demonstrated negligible acute oral toxicities for both MDI and TDI (Woolrich 1982). The oral LD50 (male rats) for TDI and MDI were determined to be 5,110 and greater than 10,000 mg/kg of body weight, respectively. Oral uptake is presumed to be an irrelevant exposure, especially in the workplace, other than trace amounts from endogenous clearing of the respiratory tract. One suicide attempt by consumption of 30-ml of two-component MDI system has been reported. Following surgical removal of a block of foam from the stomach, the individual survived with no other signs of illness.

Ocular

Application of TDI to the eyes of rabbits produced irritating effects, without any indication of percutaneous resorption (Malten and Zielhuis 1964). Corneal opacity and circumcorneal injection were observed to continue 30- and 7-days postexposure (Woolrich 1982). Rabbit eye irritation marked by inflammation and lacrimation was also observed using 10% MDI. MDI and TDI vapors and aerosols are expected eye irritants with documented acute irritation thresholds established in controlled experiments with human volunteers (Allport 2003). Symptoms from mild conjuctival irritation to severe lacrimation were noted from exposure to 80 ppb 2,4-TDI and 1300ppb 65/35 TDI for 30- and 10-minutes, respectively. Splashes have been reported and resulted in transient inflammation, impaired visual accommodation, and corneal edema.

Distribution

Kennedy et al. characterized the biochemical events initiated following inhalation of ^{14}C-labelled TDI vapors at concentrations ranging from 0.026 to 0.821 ppm for 4 hours in rats (Kennedy et al. 1994). Rapid uptake and linear correlation between exposures and blood level radioactivity were reproduced from other studies using a guinea pig model. The highest levels of radioactivity were detected in the airways, gastrointestinal system and blood, while almost 80% of the label was recovered in the plasma. Essentially 100% of the label found in plasma was amalgamated in biomoleular conjugates with molecular weights greater than 10kDa. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoretic (PAGE) characterization of these plasma retentate fractions is shown in Figure 1-18.

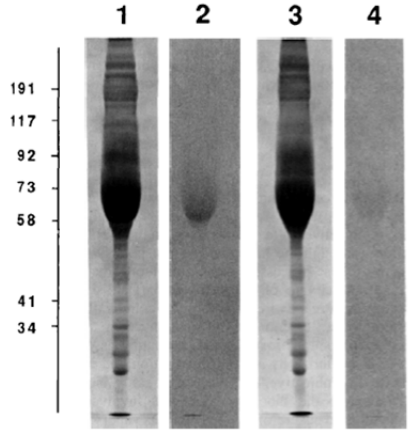

FIGURE 1-18. SDS PAGE showing plasma retentate fractions containing biomolecular conjugates (Kennedy et al. 1994).

The major fraction present represents a 70kDa molecular weight protein, putatively denoting serum albumin. Due to the high reactivity of isocyanates, a reaction with biological molecules takes place rapidly in the airway rather than passive diffusion. However, adducts of isocyanates found in proteins in the circulatory system suggest a transport mechanism.

Therefore, distribution of isocyanates is mostly governed by preceding reactions in the airway and circulates around the body as reaction products.

Irreversible bonds are formed with nucleophilic attacks by hydroxyl and amino groups in the bioglogical system at physiological pH (Brown et al. 1987). However, cellular thiols have been shown to react with isocyanates, specifically methylisocyanate and TDI, to form thiocarbmates, S-[N-(1-methyl-3,3-diphenylpropyl)carbamoyl]glutathione, that are reversible in aqueous solution through a base-catalyzed E1cB mechanism of degradation (Figure 1-19) (Baillie 1991; Bourne 1984):

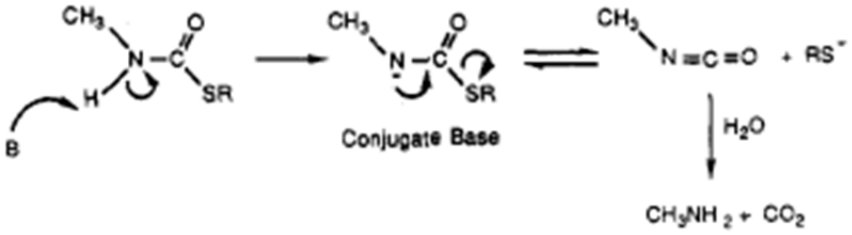

FIGURE 1-19. Degradation of thioester via E1cB mechanism (Baillie 1991).

Accordingly, glutathione, which is highly expressed in the lung, has been hypothesized to react with isocyanates, render it biologically inactive, and transport the labile residue to a distal location that is capable of reversing through the isocyanate group and reacting with a "local" nucleophile in that region (Han 1989).

TDI has been shown to increase epithelial mucous production (a hallmark of asthma), which preserves intracellular glutathione levels against TDI-mediated depletion (Lantz et al. 2001; Redlich and Karol 2002). These epithelial effects suggest celluar injury and permeability changes in the airway. However, tight junctions were perceived intact after exposure to TDI according to measurements of transepithelial resistance of airway epithelial monolayers (Lange et al. 1999).

Reisser et al. conducted stability studies between MDI and glutathione under various pH and buffer conditions to determine if the tripeptide was a masked equivalent that indirectly promoted modification at unexposed physiological sites (Reisser, Schmidt, and Brown 2002). Analytically pure mono- and bis-S-(glutathionyl) adducts with MDI were synthesized as likely candidates capable of forming under physiological conditions. The rate of degradation was proportional to the pH. Under mildly basic conditions, both compounds were unstable, and as pH increased from 7.4 to 8.2 so did the rate of reversal.

Additionally, Gledhill et al. measured radioactivity (^{14}C) in rat tissue and carcass following a post-exposure timeline of 0, 8, 24, and 168 hours to characterize the disposition of inhaled MDI (Gledhill et al. 2005). Bile duct cannulation in a cohort of rats allowed investigators to determine the proportion of inhaled MDI eliminate in the bile. A profile of tissues examined with attendant concentrations of radioactivity is shown below in Table 1-1.

Table 1-1. Tissue profile of radioactivity (Gledhill et al. 2005)

Tissue	0 h		8 h		24 h		168 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adrenals	0.267	0.117	0.292	0.114	0.237	0.080	0.281	0.052
Brain	0.019	0.006	0.014	0.004	0.012	0.003	<0.006	<0.002
Gastrointestinal tract	0.209	0.043	0.111	0.036	0.055	0.025	<0.007	<0.00(
Gonads	0.089	0.008	0.089	0.014	0.050	0.008	0.013	0.00(
Heart	0.210	0.027	0.177	0.017	0.090	0.022	0.031	0.00(
Kidneys	0.167	0.027	0.169	0.030	0.116	0.011	0.032	0.003
Liver	0.198	0.027	0.172	0.025	0.111	0.021	0.021	0.002
Lungs	6.782	1.829	5.415	1.154	2.850	0.680	1.637	0.413
Nasal tissue (o)*	0.998	0.215	0.726	0.355	0.341	0.065	0.237	0.209
Nasal tissue (r)*	66.999	80.166	394.313	321.665	16.561	27.191	2.910	0.63(
Oesophagus	0.464	0.229	0.150	0.041	0.077	0.026	<0.163	<0.18(
Pancreas	0.063	0.020	0.067	0.008	0.053	0.016	0.035	0.014
Spleen	0.094	0.012	0.097	0.020	0.076	0.011	0.042	0.00(
Stomach	0.150	0.105	0.117	0.069	0.114	0.105	0.016	0.01(
Thyroid	0.973	0.722	0.007	0.001	0.175	0.055	0.301	0.327
Trachea	2.940	3.358	1.304	0.652	1.434	1.485	0.168	0.068
Abdominal fat	0.018	0.004	0.024	0.005	0.016	0.004	<0.006	<0.00(
Bone	0.042	0.003	0.043	0.009	0.024	0.004	0.007	0.002
Muscle	0.027	0.004	0.034	0.006	0.019	0.001	<0.010	<0.007
Skin	0.074	0.027	0.067	0.019	0.078	0.024	<0.020	<0.01(
Blood	0.520	0.287	0.323	0.069	0.188	0.025	0.114	0.047
Plasma	0.547	0.060	0.360	0.186	0.183	0.035	0.041	0.003
Residual carcass	0.120	0.033	0.114	0.025	0.063	0.014	0.016	0.004
Stomach contents	<0.026	<0.041	<0.075	<0.084	0.012	0.009	<0.003	<0.00
Gastrointestinal contents	0.321	0.042	0.433	0.151	0.119	0.060	0.006	0.001

Values are µg equivalents of MDI g^{-1} tissue. Each value is a mean of four rats with standard deviation (SD).
*r, respiratory; o, olfactory.

While a wide-distribution and tissue sequestration of radioactivity is evident from this study, cumulative excretion of radioactivity in urine and feces between rats with and without bile-duct cannulation suggests that this distribution pattern is more in line with ingestion rather than inhalation, which is the occupational exposure of concern.

Due to the high content of albumin in plasma and glutathione in the lung, reactions with these biomolecules seem most plausible with subsequent distribution (Gledhill et al. 2005; Kennedy et al. 1994).

Metabolism

The metabolic fate of MDI and TDI has been implicated in genotoxic effects via bioactivation to their respective arylamines and *N*-hydroxy arylamines. Briefly, hydrolysis and decarboxylation of MDI yields methylenedianiline (MDA), which can further be metabolized by mixed function monooxygenases to *N*-hydroxy arylamines, *N*-sulfonyloxy arylamines, *N*-acetoxy arylamines, or *N*-hydroxy arylamine *N*-glucuronides (Sabbioni et al. 2000).

Vock et al. proposed a possible procarcinogen role, in relation to the above transformation reactions, of inhaled MDI in the upper respiratory tract of rat as a result in their investigation to detect derived DNA adducts using ^{32}-P-postlabeling methods (Vock et al. 1996). MDA-DNA adducts were detected only in the olfactory epithelium as opposed to the lung (i.e., target organ for MDI tumors), as well as systemically. These results differed from a previous study where MDI was topically administered on rat skin, which demonstrated formation of isocyanate-DNA adducts in DNA of epidermal cells (Vock et al. 1995). The exposure dose in this study was 3mg/m^3, which may not reflect realistic workplace conditions. However, isocyanate concentration and duration of exposure are intimately linked to absorption and the extent by which they are capable of exerting their effects. Breakdown intermediates in the

proposed GSH-mediated transport mechanism also provide suitable conditions for metabolism of MDI. In the Reisser et al. study, MDI was reacted with excess GSH in a biphasic system (water/toluene) under quasi-physiological conditions to evaluate the presence of a likely monoadduct, *S*-({[4-(4'-aminobenzyl)-phenyl]amino}carbonyl)-glutathione (Reisser, Schmidt, and Brown 2002). The monoadduct was concluded to form in low levels as an intermediate in a pH-dependent degradation of another adduct. Subsequently, acetylation of the free aromatic amine of the monoadduct would promote formation of either direct isocyanate adduct formation, or indirectly AcMDA adducts with hemolglobin. However, investigators concluded that under true physiological conditions, inhaled MDI most likely forms a bis adduct, methylene-bis-(*S*-{[(4-phenyl)amino]carbonyl)gluthathione, in relation to MDI's reactivity and high levels of GSH present in the epithelial lining fluid.

The reaction pathway of arylamines and isocyanates is illustrated in Figure 1-20, which highlights the challenge in understanding the etiology of the health effects associated with isocyanate exposure (Kumar, Dongari, and Sabbioni 2009).

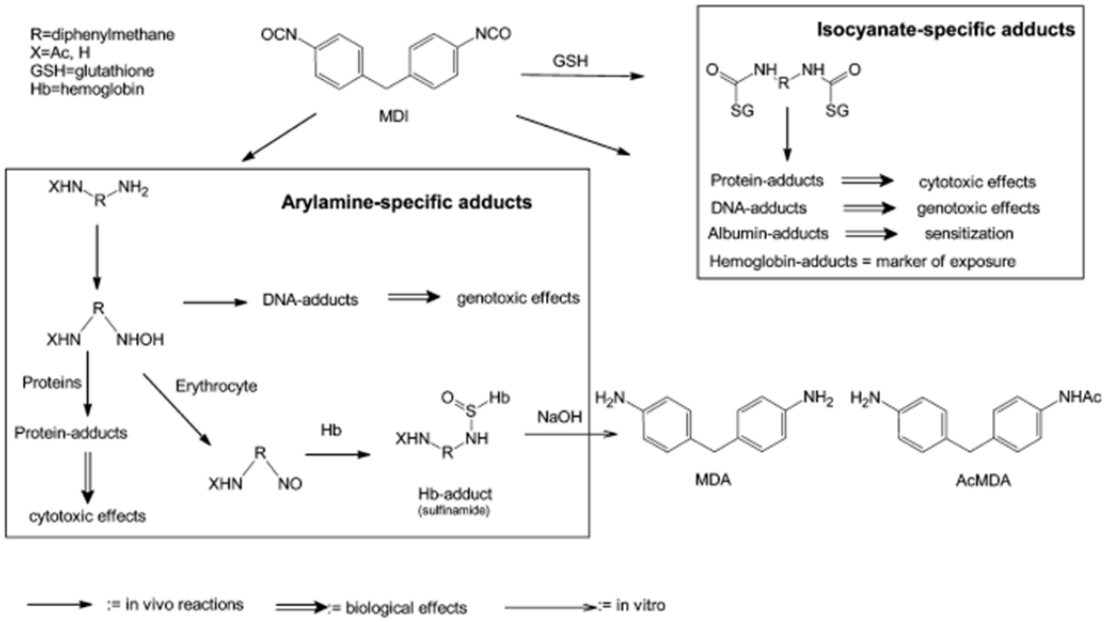

FIGURE1- 20. Reaction pathway of arlyamine and isocyanates (Kumar, Dongari, and Sabbioni 2009).

Sabbioni et al. developed a procedure to distinguish between arylamine, specifically MDA and AcMDA, and MDI adducts in hemoglobin of exposed rats (Sabbioni et al. 2000). Since protein adducts may be involved in lung sensitization and asthma, determination of which compound most likely contributes to adduct formation is important, especially for biomonitoring (see Biomarkers). *N*-acetyltransferases have been found in erythrocytes, which may introduce an acetyl group in MDI when bound to hemoglobin. Accordingly, AcMDI was synthesized and reacted with the tripeptide valyl-glycl-glycine. The *N*-terminal amino acid of valine represents the target site of adduct formation on hemoglobin (Figure 1-21)

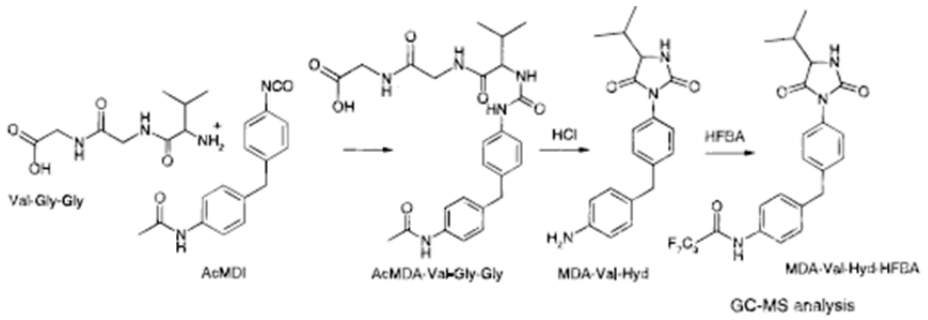

FIGURE 1-21. Reaction scheme for synthesis isocyanate adducts AcMDA-Val-Gly-Gly and MDA-Val-Hyd (Sabbioni et al. 2000).

Identification of isocyanate adducts with hemoglobin in rats chronically exposed to MDI aerosols was facilitated by gas chromatographic analysis of these synthetic compounds. Using acidic and basic hydrolysis to release either MDI or MDA from the carrier molecule, respectively, higher amounts of MDA-Val-Hyd were detected at levels 12 times higher than that of either MDA or AcMDA. The MDA-Val-Hyd is an isocyanate-specific adduct, indicating that higher amounts of these adducts should be expected in exposed workers. AcMDA and MDA were detected in this study, most likely from a sulfinamide adduct. However, arlyamine hydrolysis of MDI to MDA is unlikely, as a concerted reaction of hydrolysis and acetlyation would need to occur on one end of the isocyanate group while the other group remained unchanged in order to form a covalent bond with hemoglobin. Therefore, arylamine hydrolysis of MDI to MDA was concluded to be an insignificant metabolic pathway in rats, and the presence of diamine is a cleavage product from aggressive hydrolysis of an isocyanate-specific adduct.

In the Gledhill et al. inhalation study using [^{14}-C]-MDI, a total of five metabolites were identified in urine, bile, and feces (Gledhill et al. 2005). However, the authors were unable to distinguish the origination of the metabolites, i.e. renal, hepatic, or pulmonary. Biotransformation of this parent compound was concluded to follow two possible routes: (1) spontaneous polyurea formation, and (2) enzyme-mediated metabolism of systemically available

MDI or MDI-derivatives. *N*-acetylation of MDI was proposed to precede oxidation due to detection of *N*-acetylated, and *N*-acetylated-hydroxylated products. The presence of MDA was not detected in any of the biomatrices analyzed. A proposed metabolic pathway for MDI is presented in Figure 1-22.

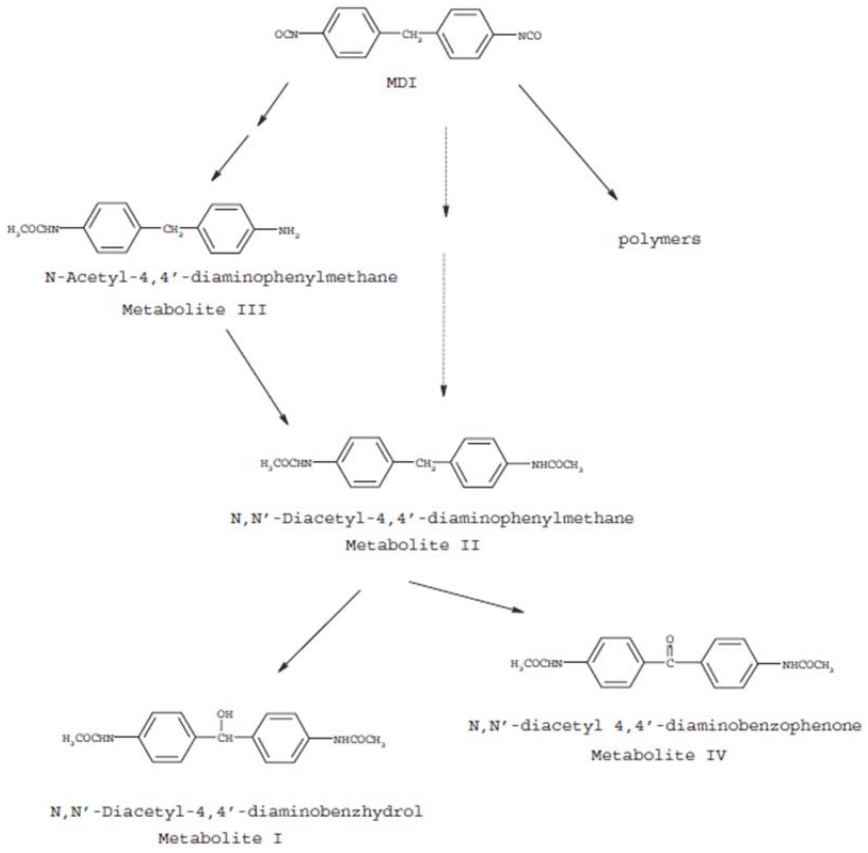

FIGURE 1-22. A proposed metabolic pathway of MDI (Gledhill et al. 2005).

Due to the prevailing conditions in the respiratory tract and inherent reactivity of MDI and TDI, hydrolysis to corresponding diamines is essentially negligible (Allport 2003). Minor formation of amines may occur with increased residence time in the bronchiolar/alveolar region, but excess diisocyanate will react immediately with the amine to form a urea and/or polyurea (Figure 1-23).

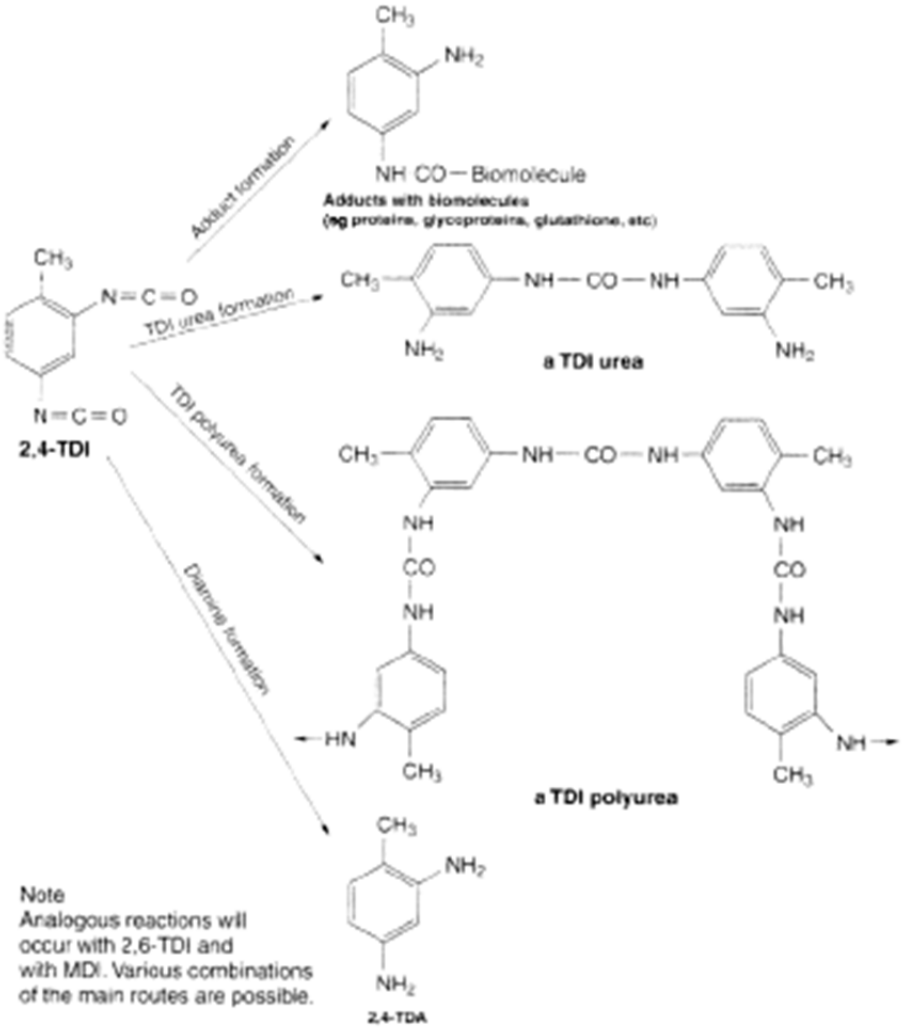

FIGURE 1-23. Reaction pathway for TDI polyurea (Allport 2003)

Therefore, reactions with biomolecules tendering amino, hydroxyl or thiol groups occur rapidly, but proteins predominantly serve as the chief reactant to which isocyanate conjugates are formed. Amino groups of proteins are irreversibly bound to isocyanates, and the bifunctionality of MDI and TDI permit intra- and inter-cross-linking of molecules containing the aforementioned functional groups (Figure 1-24).

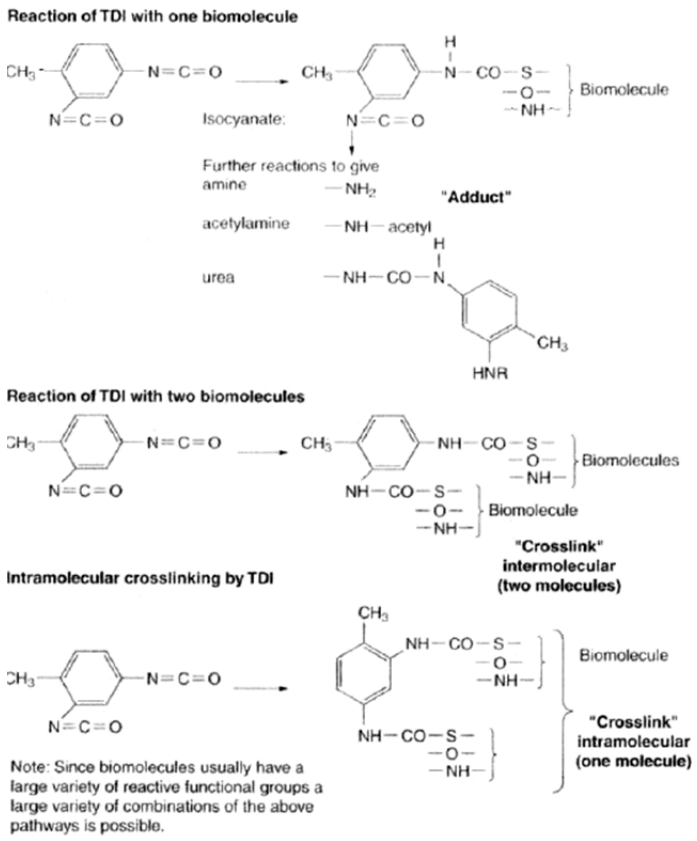

FIGURE 1-24. Isocyanate reactions with biomolecules forming intra- and intermolecular linkages (Allport 2003).

Excretion

Reactive functional groups in proteins, sugar moieties, and other biological macromolecules are ubiquitous in the body, which increases the number of possible substrates that react with isocyanates—providing a matrix of possible metabolites (Allport 2003). However, reactions with these biological monomers typically render the isocyanate biologically inactive in the form of high molecular weight conjugates, which are removed from the body by normal metabolic pathways (Allport 2003). The exceptions are antigenic conformational changes that contain neoepitopes recognized by the immune system, which is the basis for

sensitization (see next section Sensitization and Immunology) (Wisnewski, Liu, and Redlich 2010).

Inhalation studies using radiolabeled techniques have detected diisocyanate metabolites in urine, feces, and bile (Allport 2003; Gledhill et al. 2005; Kennedy et al. 1989; Kennedy et al. 1994; Timchalk, Smith, and Bartels 1994). Therefore, excretion of MDI and TDI is almost exclusively in either the urine or feces, depending on the route of exposure; there is no evidence of diisocyanate elimination through exhaled metabolic products in CO_2. In the Gledhill et al. study, 80% of the received dose was excreted in the feces, which indicates oral ingestion of MDI rather than exclusive inhalation (Gledhill et al. 2005). Analysis of urine samples over a 48 hour interval demonstrated that 12% of the dose was excreted. Other studies using radiolabeled TDI detected up to 15% of the total radioactivity recovered in urine (Allport 2003). Over 90% of the urinary metabolites identified in these studies were conjugated; consequently, no diisocyanate is excreted unchanged.

MDI and TDI Health Effects and Senistization and Immunulogy

Occupational exposures to MDI and TDI elicit serious worker health effects through interplay of inflammatory-, and immune-mediated mechanisms (Rom 1998). Inhalation exposures to these congeners are irritating to tissues, especially mucous membranes. Workers may experience mild symptoms of occupational rhinitis characterized by episodic work-related sneezing, nasal discharge, and nasal obstruction; as well as eye irritation, coughing, and short of breath (Allport 2003; Merchant et al. 1986). Neurologic sequelae, including euphoria, ataxia, mental aberrations, and headache, have also been recorded from acute and chronic isocyanate exposures, however, these symptoms are mostly subjective without any predictability (Karol 1986; Bardana, Montanaro, and O'Hollaren 1992; Registry 2008).

Skin exposure to isocyanates may cause contact dermatitis (both irritant and allergic) with symptoms such as rash, itching, hives, and swelling of extremities (Bello et al. 2004; Streicher 1994). Additionally, growing evidence in current literature strongly suggests that dermal exposure plays a pivotal role in sensitization and asthma development (Bello et al. 2004).

Occupational asthma, an inflammatory disorder of the airways, is the most prevalent respiratory illness attributable to MDI and TDI exposure (Rom 1998). These low-molecular weight compounds may induce allergic and/or irritant asthmatic responses that are with, and without a period of latency, respectively, which is schematically illustrated in Figure 1-25 (Rom 1998; Pronk et al. 2007).

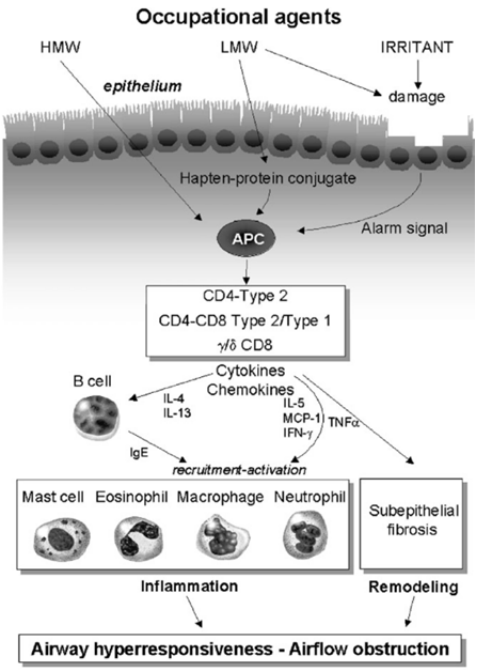

FIGURE 1-25. Overview of the mechanisms involved in the pathogenesis of occupational asthma mediated by both high molecular weight and low molecular weight agents (Mapp et al. 2005). As shown in this figure, asthma is a heterogeneous disease with respect to immunopathology and environmental causes (Mapp et al. 2005; Holgate 2008; Borish and Culp 2008).

Other adverse effects to the respiratory system include hypersensitivity pneumonitis (HP), and reactive airways dysfunction syndrome (RADS), and possibly accelerated pulmonary function loss (Allport 2003; Pronk et al. 2007; Wegman et al. 1977; Leroyer et al. 1998; Schreiber et al. 2008; Baur 1995).

Asthma

Asthma is an inflammatory disorder characterized by episodes of bronchoconstriction and excessive mucous secretions, which induce coughing, wheezing, tightness of the chest, and labored breathing, which is illustrated in Figure 1-26. (Allport 2003)

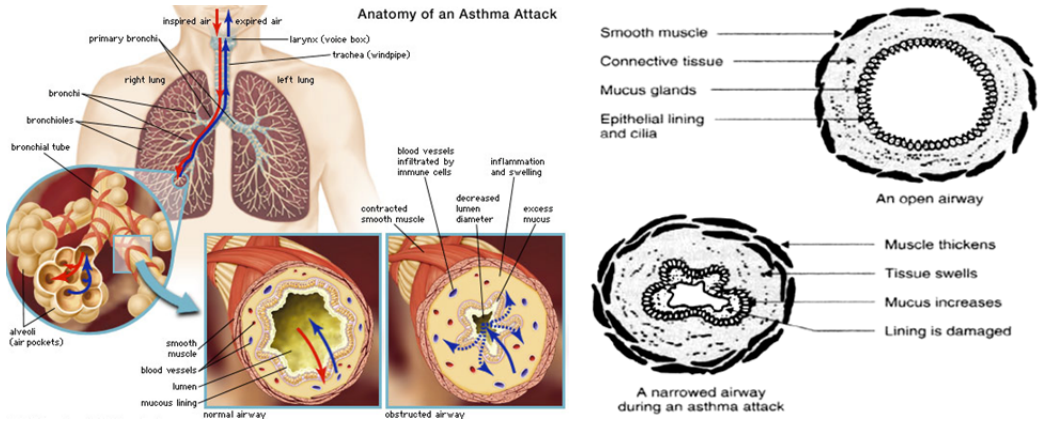

FIGURE1- 26. Anatomy of asthma attack and its effect on the airways (Allport 2003; Encyclopedia 2001).

Asthma is the most prevalent work-related respiratory disease in industrialized countries with an estimated morbidity of 130,000 to 975,000 physician visits, 10,000 to 75,000 hospital admissions, and a conservative 20,000 to 150,000 days' work lost per year (Cartier 1994; Rom 1998). These statistics have considerable social and economical implications for employers and governmental agencies because of increased workers' compensation costs and decreased productivity as a result of lost workdays, job reassignments, and training new workers. In fact,

the most recent estimates indicate that in the U.S., the direct medical costs for occupational asthma are over $1.6 billion (Leigh et al. 2002).

Asthma is a distressing disease with debilitating and disabling consequences that compromise workers safety (e.g., on ladders or in confined spaces) and ultimately impacts the patient's quality of life, including his or her family (Merchant et al. 1986; Allport 2003). Current estimates indicate that 11 million workers in various workplaces are exposed to at least one known agent associated with work-related asthma.

Work-related asthma is an umbrella term that groups together both occupational asthma and work-aggravated asthma (Mapp et al. 2005). This latter type of asthma is a pre-existing condition worsened by irritants, such as MDI and TDI, or physical stimuli in the workplace (Rom 1998). Concurrent asthma, or other mimicking symptoms that are aggravated by work or the work environment does not establish a causal relationship between workplace exposure and asthma, nor discriminate a causal agent in the workplace from the myriad of nonoccupational causes; as a result, workers may not require extreme work restrictions through better medical management and slight reduction of workplace exposures (Mapp et al. 2005; Tarlo and Liss 2002). Conversely, occupational asthma is provoked by inhalation of workplace agents, which is a key differential in the diagnosis, with subsequent episodic airflow limitation usually accompanied by bronchial hyperresponsiveness (Merchant et al. 1986; Rom 1998). Two types of occupational asthma can be distinguished according to the presence or absence of a latency period prior to the development of asthma (Rom 1998; Chan-Yeung and Malo 1995; Mapp et al. 2005). The most common type occupational asthma is with a latency period, which may develop after a period of a few weeks to several years (Chan-Yeung and Malo 1995). This type of

asthma encompasses allergic/immunologic mechanisms, whereas the second type of occupational asthma develops without a latency period by nonimmunologic mechanisms.

During the latent period, the worker may remain asymptomatic while the exposure continues and the allergy evolves (Rom 1998). The heterogeneity of mechanisms involved with the physiological abnormalities of asthma eventually culminate into variable or chronic airflow limitation, characterized by a prolonged forced expiratory time attributed to bronchoconstrictor mediators and remodeling with structural changes (Hargreave and Parameswaran 2006; Association 1997). Specific and nonspecific airway hyperresponsiveness co-manifest in parallel with work-related airflow limitation, and is not a predisposing host factor for initiation of occupational asthma (Rom 1998). The specific airway hyperresponsiveness relates to allergic/immunologic influences, while insult to the bronchial mucosal and airway inflammation account for nonspecific airway hyperresponsiveness.

Inflammation is essentially localized in the lung region, especially from prolonged repeated inhalation exposure, which the effects may be severe and even fatal (Allport 2003). For instance, in 2003, a work-related fatality occurred from an acute asthmatic reaction following exposure from spraying an isocyanate-containing bedliner (Chester et al. 2005). Inhalational exposures to excessive amounts of isocyanates may precipitate the onset of diffuse alveolar damage, and ultimately acute respiratory distress syndrome (ARDS) (Rom 1998). Disruption of airway epithelium from exposure to high vapor concentrations promotes infiltration of mast cells and eosinophils, key players in the inflammatory response (Raulf-Heimsoth and Baur 1998).

High concentrations of diisocyanates, in experimental models only, have triggered neurogenic inflammation through activation of the efferent function of capsaicin-sensitive sensory nerves and the inhibition of neutral endopeptidase concurrently exerting

bronchoconstriction on bronchial smooth muscle (Sastre, Vandenplas, and Park 2003; Mapp et al. 1998; Scheerens et al. 1996).

Depending on the exposure scenario (e.g., sensitized worker and exposure levels) an asthma attack may occur with immediate, latent, dual onset (both immediate and latent components after exposure), or continuous symptoms (Rom 1998; Allport 2003; Streicher 1994). Once a worker has been sensitized (e.g.,, by MDI or TDI), exposures to exceedingly low concentrations, even below occupational exposure limits or standards, may produce a life-threatening asthmatic reaction, which then makes exposure control almost impossible (Redlich and Karol 2002). Essentially, the worker should be completely removed from potential exposures, and relocated to a new job or job site (Bardana, Montanaro, and O'Hollaren 1992).

Different groups and institutions have propounded the definition of occupational asthma to advocate for different standards and principles (e.g., medico-legal vs. surveillance) (Rom 1998). For example, a definition may stress allergic pathogenesis, such as, "variable airflow limitation caused by sensitization to a specific agent encountered at work and excluding other occupational causes of variable airflow limitation not due to sensitization" (ACCP 1990). However, the term sensitizing agent needs a straightforward medico-legal definition, especially for litigating purposes in compensation claims, or determining disability versus impairment (Chan-Yeung et al. 2003). Therefore, in guidelines published by the American Thoracic Society, occupational asthma is defined as asthma caused by a specific workplace exposure and not to influences outside the workplace (Merchant et al. 1986; Rom 1998).

There are over 200 different agents capable of initiating occupational asthma that the worker either manufactures, encounters directly, or incidentally (Rom 1998). Presently, diisocyanates are most frequently reported cause of chemically induced occupational asthma

(Redlich and Karol 2002). The European Union has labeled both MDI and TDI as R-phase R 42, which is defined as "may cause sensitization by inhalation" (van Kampen, Merget, and Baur 2000). Prevalence estimates of sensitization and asthma, the primary health concerns in the exposed workforce, have varied slightly depending on the operation. For instance, Bello et al. have reported 1-20% prevalence in exposed worker populations while Streicher et al. have reported 5-10% in production facilities to 25% in polyurethane production plants and 30% in polyurethane operations (Bello et al. 2004; Streicher 1994)

The term asthma may be modified by words or phrases indicating its etiology and factors provoking attacks; accordingly, diisocyanate asthma is a more specific term that delineates the unequivocal causal relationship between MDI/TDI exposure and occupational asthma (Bardana, Montanaro, and O'Hollaren 1992; Merchant et al. 1986). This is an appropriate distinction because, for example, diisocyanate-induced asthma was shown to account for 57% of all claims accepted by a Canadian provincial Workplace Safety and Insurance Board for occupational asthma (Tarlo et al. 1995). Accordingly, practical and effective controls can be implemented specific to the diisocyanate exposure to protect workers' health.

The natural history and outcome of disease has been resolved, typically, by cessation of exposure to MDI and TDI (Bardana, Montanaro, and O'Hollaren 1992). However, diisocyanate-induced asthma is associated with immediate and late reactions, which complicates recognition and diagnosis of the symptoms, especially without a comprehensive occupational history (Bardana, Montanaro, and O'Hollaren 1992; Merchant et al. 1986). Immediate reactions may last for several hours or until leaving work, but are reversible using isoproterenol. Generally, late reactions develop slowly and progressively get worse even after several years removed from exposure. The majority of workers/patients with latent occupational asthma suffer from

permanent impairment and disability (Chan-Yeung and Malo 1995). Yeung and Malo created an algorithm for clinical investigations of occupational asthma, as seen in Figure 1-27.

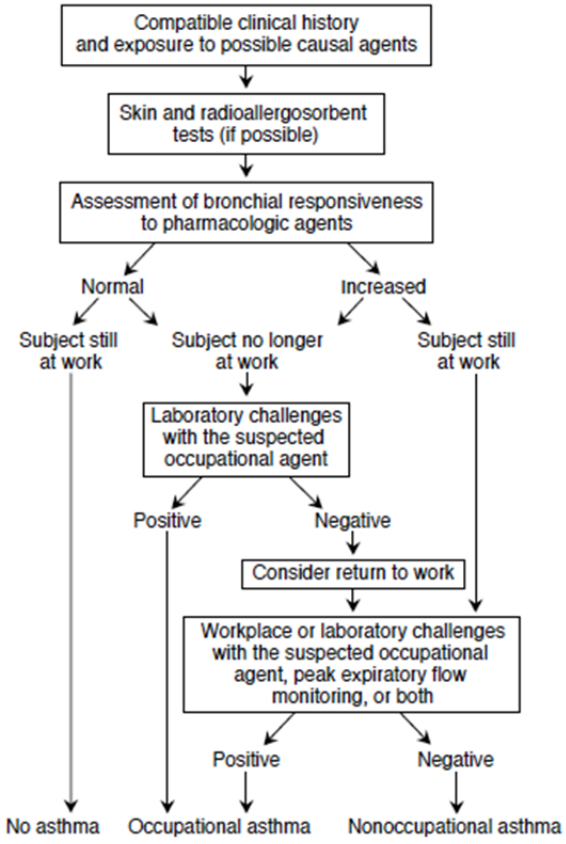

FIGURE 1-27. Flowchart of algorithm for diagnosing occupational asthma (Chan-Yeung and Malo 1995).

Additionally, diisocyanate asthma has shown unique features, such as a: significantly earlier onset of asthma symptoms compared to occupational asthma caused by other agents (mean 5 years vs. 7 years); younger population affected; and, greater likelihood to be atopic and have smoked (Tarlo and Liss 2002). Atopy and smoking are putative risk factors linked to the development of asthma and its severity (Allport 2003; Mapp et al. 2005). Atopy is an inherited disposition for allergic diseases associated with elevated levels of total IgE antibodies in the serum, mainly in workers exposed to high-molecular-weight agents (e.g.,, animal proteins), which may cause skin reactivity.

Cigarette smoking has been reported to be associated with the development of occupational asthma in certain cohorts of exposed workers (e.g.,, operations involving platinum salts or anhydride compounds), by means of interacting with both high and low levels in inducing sensitization and altering cellular composition of airway mucosa (Mapp et al. 2005). Additionally, cigarette smoke increases the risk of IgE sensitization to high-molecular-weight agents that cause occupational asthma.

Accordingly, atopy and smoking are not confounders in the induction of diisocyanate asthma. Furthermore, IgE responses have frequently been undetected in the serum of workers, and represent only a minority of cases of ioscyanate-induced asthma (Mapp et al. 2005; Campo et al. 2007; Allport 2003; Chan-Yeung et al. 2003). Therefore, atopy is weak predictor of sensitization and development of diisocyanate asthma (Allport 2003; Mapp et al. 2005; Mapp et al. 1988). Additionally, smoking habits of asthmatics in six diisocyanate-exposed populations were not associated with greater risk of diisocyanate-induced asthma than non-smokers. Generally, the temporal relation between immunologic occupational asthma and cigarette smoking significantly differ in severity of disease. For instance, while mild symptoms of occupational asthma may be experienced during time of diagnosis, a greater severity of disease is more likely associated with cigarette smoke (Moscato et al. 2002).

A multitude of biomolecular outcomes may initiate respiratory morbidity through irritative and antigenic effects due to bifunctional capabilities of diisocyanates that allow intra- or intermolecular linkages. The development of an asthmatic condition may initially develop from an acute exposure, but sensitization (production of antibodies) typically ranges from a few months to several years of exposure (Streicher 1994). Frequent exposure to large spills of diisocyanates is widely accepted as a predisposing factor to clinical manifestion of diisocyanate

asthma, as shown in a cohort of 332 TDI workers, but specific mechanisms that lead to sensitization were not identified (Rom 1998; Scheel, Killens, and Josephson 1964; Brooks 1982).

Potential cellular and biochemical mechanisms of sensitization have been studied, including pharmacological, immunological, and nonimmunological (irritant) in attempts to elucidate sensitization events that lead to diisocyanate-induced asthma (Bardana, Montanaro, and O'Hollaren 1992). Direct pharmacologic action on the airways may explain the pathogenesis of asthma by nonimmunologic release of histamine and other spasmogens (Merchant et al. 1986). For instance, TDI has been postulated to interfere with autonomic control of airway tone. Butcher et al. demonstrated isocyanates as pharmacologic inhibitors *in vitro* with downstream effects on bronchial tone consistent with the β-adrenergic blockade theory of asthma (Davies et al. 1977). Briefly, TDI behaved as an antagonist, blocking β-adrenergic receptors on lymphocytes, in turn reducing their ability to produce cyclic adenosine monophosphate (cAMP) at levels necessary to maintain bronchial tone. However, this study received much criticism for use of dimethyl sulfoxilide, which altered the phospholipid characteristics of the cell membrane that increased receptor sensitivity to TDI.

Immunologic and nonimmunologic causation of diisocyanate-induced sensitization have been the central focus of mechanistic studies due to the unpredictability in clinical diagnoses and need for medical surveillance. Immune-mediated mechanisms induced by exposure to MDI and TDI are variable and complex, such that the same antigenic exposure can cause disease or immune tolerance (Bello et al. 2004). Furthermore, low-level exposures may be more immunogenic and pathogenic than higher doses. In fact, Herrick et al. demonstrated greater airway inflammation in a novel mouse model of diisocyanate-induced asthma in skin sensitized mice using a lower dose of isocyanate following an airway challenge (Herrick et al. 2002).

Vanoirbeek et al. developed a chemical-induced asthma animal model to better characterize immunological responses and determine persistence of systemic and ventilatory responses fundamental to TDI-induced asthma (Tarkowski et al. 2007; Vanoirbeek et al. 2009; Vanoirbeek et al. 2008). Briefly, BALB/c and severe immunodeficiency disease (SCID) mice received dermal application of TDI on days 1 and 7 followed up by an intranasal challenge on day 10 with nonirritant amounts of TDI, which reproduced classic features of human asthma.

Cellular profiles from bronchoaveoloar lavage (BAL) performed after various time points of intranasal instillation showed an influx of neutrophils, tumor necrosis factor-α, and macrophage inflammatory protein (MIP)-2, which persisted up to 50 days after initial treatment. Significantly elevated levels of total serum IgE, IgG1, and IgG2a were detected in blood drawn from the retroorbital plexus that remained until 90 days after sensitization.

Other measured parameters included lymphocyte populations in auricular and cervical lymph nodes, and IL-4 and IFN-γ levels in lymph node cells. Relatively, CD19+ cells were increased while CD4+ and CD8+ cells in the auricular lymph nodes decreased. IL-4 and IFN-γ release were amplified in auricular lymph node cells only until 20 days after sensitization. Results from these studies showed that the reactions are lymphocyte dependent, but without preferential stimulation of either Th1 or Th2 lymphocytes.

Ventilatory function was assessed using whole body plethysmograph under resting conditions and immediately following intranasal instillation. Every 30 seconds the enhanced pause (Penh) was calculated to determine airway obstruction in these TDI treated mice. Briefly, Penh is a dimensionless, composite index that is sensitive to changes in the shape of the airflow pattern as the animal breathes (Frazer, Reynolds, and Jackson 2011). Increases in Penh were suggestive of airway obstruction. Both ventilatory and inflammatory responses were inversely

related to time between sensitization and challenge, despite lasting humoral signs of sensitization. This was highlighted in a follow-up study with multiple challenges (up to six challenges with one week recovery between challenges) of intranasal instillation of TDI.

Increasing challenges seemed to induce a tolerance to TDI evident by diminished presence of markers such as total cell count and neutrophils, and decreased magnitude of Penh. However, authors concluded that Th2 stimulation was achieved in this study by the increased % of B cells present and total serum IgE levels, which plateau and remained constant until six intranasal challenges.

Non-immunologic processes include repetitive injury-repair cycles and oxidative stress, which may modulate isocyanate responsiveness, including the development of asthma (Lantz et al. 2001; Mishra et al. 2010; Mishra et al. 2009). Repetitive cycles of injury and repair has been suggested to play a direct role in diisocyanate asthma pathogenesis while immune sensitization and development of specific IgE is a secondary event (Wisnewski and Jones 2010).

In vitro experiments combined with molecular techniques using subcytotoxic isocyanate concentrations have shown a variety of pro-inflammatory effects (Wisnewski et al. 2002; Sastre, Vandenplas, and Park 2003). Analytical techniques have also shown isocyanate capabilities to disrupt oxidant homeostasis through reducing intracellular glutathione, activation of mitogen-activated protein kinase, and production of RANTES (regulated on activation, T-cell expressed and secreted) (Wisnewski and Jones 2010; Lantz et al. 2001; Hashimoto et al. 2001). Expression and upregulation of adhesion markers on monocytic cells together with increased intracellular peroxide and reactive oxygen species from diiscoyanate exposure may potentiate the infiltration and adhesion of inflammatory cells at the site of exposure (Sastre, Vandenplas, and Park 2003; Elms et al. 2001). The capability of isocyanates to induce reactive airway disease syndrome (see

RADS section) and asthma immediately following a single exposure (e.g.,, large spills), fortifies a cytotoxic role in the development of isocyanate hypersensitivity.

Since clinical manifestations of sensitization are characteristic of diisocyanate asthma, the role of nonimmunologic mechanisms remains to be defined. While some reports suggest that chemical-induced toxicity represent underlying pathological mechanisms, others postulate an indirect role in the development of asthma by amplifying immunologocial responses to MDI and TDI (Wisnewski and Jones 2010; Sastre, Vandenplas, and Park 2003).

Structure-biological activity relationship models have furthered understanding the mechanisms of isocyanate sensitization (Karol, Macina, and Cunningham 2001). These models have predicted that the inherently high reactivity of the NCO functional group is a key physico-chemcial property that discriminates sensitizing agents from non-sensitizers (Karol, Macina et al. 2001; Wisnewski and Jones 2010). However, even after decades of research the pathogenesis of diisocyanate (MDI or TDI)-induced asthma remains a contemporary, and controversial debate due to what appears as a multifactorial etiology, specifically focusing on whether or not immunological mechanisms are IgE-dependent or IgE-independent (Wisnewski et al. 2011).

IgE--mediated hyspersensitivity

Clinical features of diisocyanate asthma indicate that cell-mediated immune responses seem to play an important role in the pathogenesis, most notably resembling Type 1 Immune Hypersensitivity, or allergic asthma (Raulf-Heimsoth and Baur 1998; Wisnewski and Jones 2010). A fundamental feature of Type 1Immune Hypersensitivity is an isotype class switching of allergen specific immunoglobins to the epsilon constant region, for instance, producing immunoglobin E (IgE) (Wisnewski and Jones 2010).

Dendritic cells are the most efficient antigen-presenting cells in the lungs capable of activating CD4+ T cells (Th0) by engaging with T-cell receptors via MHC class II-peptide complex composed of allergic peptides (Raulf-Heimsoth and Baur 1998; Frew 1996; Wills-Karp and Ewart 2004). Subsequent to activation, T cells proliferate and secrete cytokines, which promotes improved response time, "immunological memory", and regulates the immune response (Raulf-Heimsoth and Baur 1998). Differentiation of the naïve T cell to either Th1 or Th2 subtypes depends on the nature of antigen (e.g.,, allergen or mycobacteria), resident cell cooperation (e.g.,, mast cell or NK-cells), and cytokines secreted (IL-12 or IL-4) (Figure 1-28). Non-atopic, non-asthmatic individuals are susceptible to T-cell differentiation towards a Th1 pattern, which is consistent with a pattern of cytokine secretion that includes interferon-γ and IL-2 (Wills-Karp and Ewart 2004).

Th2 phenotype is associated with IgE and eosinophilia, and consequently with allergy. A panel of cytokines is secreted from activated Th2 cells that are essential for T-cell survival and expansion, which include enhanced production of IL-4, IL-10, IL-5, and GM-CSF (Wills-Karp and Ewart 2004; Raulf-Heimsoth and Baur 1998). IL-13 is also characteristic of the Th2 response, which plays an essential role in pathogenesis by activating an IL-4Rα and IL-13Rα1 receptor complex. Activation of this receptor complex along with newly discovered genes C5 (complement factor 5) and TIM1 (T-cell immunoglobin and mucin-domain containing molecules) are recent discoveries that have bridged the gap in understanding polarization to Th2 differentiation in asthmatic individuals (see Genetics) (Wills-Karp and Ewart 2004). Additionally, IL-4 and IL-10 promote the production of Th2 cytokines (including auto-regulation) and inhibition of a variety of other cytokines (e.g.,, IFN-γ and IL-12), respectively.

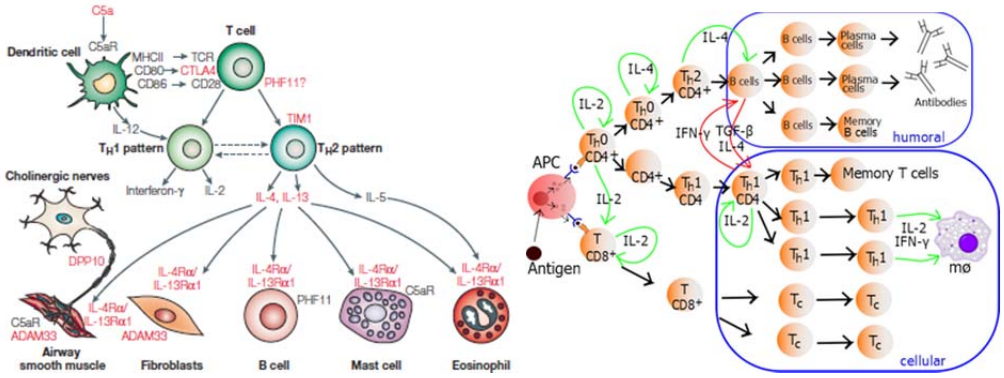

FIGURE 1-28. Differentiation to Th1 and Th2 subtypes (Rang 2003) (Wills-Karp and Ewart 2004).

IL-4 is the first essential cytokine signal in isotype switching to IgE, inducing RNA transcription at the Cε locus via stimulation of STAT6 (Raulf-Heimsoth and Baur 1998; Paul 2008). IL-4 engages I IL-4 receptor, which initiates JAK kinase phosphorylation of tyrosine residues in the intracellular domains of this receptor, specifically IL-4Rα and IL-13Rα1. Subsequent phosphorylation of STAT6 molecules forms homodimers that translocate to the nucleus and bind to a specific promoter region. Other transcription factors such as NF-κB are also important downstream players of increasing expression of Cε germline transcripts, which all facilitate formation of a functional IgE ε heavy chain, which define this specific antibody.

Chiung et al. demonstrated the release of IL-4 were linked to an increase in cytosolic $[Ca^{2+}]_c$ in several airway cell lines treated with TDI (Chiung et al. 2010). Cell lines included: human epithelial cell line H1355; human T-cell line Jurkat; and human neuroblastoma SH-SY5Y cells; as well as primary cell cultures of white blood cells. Calcium mobilization accounting for increased concentrations most likely occurred from both intracellular storage and extracellular influx. Dose-dependent stimulation of cytokine release was detected within the first five minutes of exposure, suggesting a sentinel role in the early inflammatory responses of atopic asthma.

Following production, IgE antibodies circulate in the blood and organs, and cause mast

cell degranulation in newly sensitized individuals. Consequently, inflammatory reaction, vasodilation, and bronchoconstriction result from mediators that are either preformed (e.g.,, histamine, tryptase, etc.) or newly generated (e.g.,, leukotreines, prostaglandins). (Raulf-Heimsoth and Baur 1998)

Other key features of diisocyanate asthma with similarities to IgE-mediated hypersensitivity, include: asymptomatic latency period, a small percentage develop illness, acute and delayed reactions, and bronchial hypersensitivity that worsens and recovers in relation to exposure (Rom 1998; Wisnewski and Jones 2010; Campo et al. 2007; Redlich and Karol 2002). Generally, the latency period ranges from months to years before diisocyanate asthma develops (Malo and Chan-Yeung 2009). This time period combined with immediate and dual phase reactions to MDI and TDI exposure reflect the complex and diverse spectrum of systemic immune sensitization (Maestrelli et al. 2009).

Microscopic anatomical changes consistent with histopathologic features observed in allergic asthma were present in airway biopsies from diisocyanate-induced asthma patients, including submucosal inflammatory infiltrate in the bronchial wall characterized by increased numbers of eosinophils, T lymphocytes, and mast cells (Figure 1-29). (Redlich and Karol 2002) Epithelial desquamation, subepithelial fibrosis, and goblet cell hyperplasia were also visible, which accounts for mucous hypersecretion within the airways.

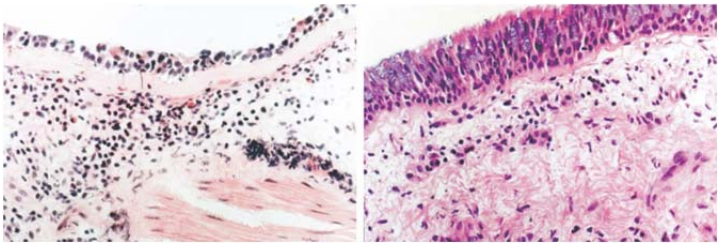

FIGURE 1-29. H&E stain comparison of diisocyanate asthma (Left) and control (Right) in a bronchial biopsy specimen. Hallmark proliferation of T lymphocytes and eosinophils with presentation of epithelial desquamation, subepithelial fibrosis, and submucosal inflammatory infiltrate are seen in Left image, consistent with atopic asthma (Redlich and Karol 2002).

BAL and sputum specimens of isocyanate-induced asthma patients have confirmed the presence of immune cells, notably CD4+ T cells, and cytokines IL-4, IL-6, and IL-13 (Fisseler-Eckhoff et al. 2011). Cells expressing IL-5 messenger RNA have also been isolated from bronchial biopsies, which are fundamental in regulating eosinophil. Elevated concentrations of IL-5 are a good predictor of activation markers of T-lymphocytes and eosinophil, suggesting an interaction between these two cells. This interaction may play an important role in the pathogenesis of IgE dependent asthma.

However, detection of circulating specific IgE antibodies is intermittent in diisocyanate exposed workers (Rom 1998). For instance, detectable levels are found in approximately 5-30% of symptomatic workers, and less than 1% of exposed asymptomatic workers, which may be the result of inappropriate methods, insufficient assay sensitivity or specificity, or improper isocyanate antigen utilized in the immunoassay (Table 1-2) (Encyclopedia 2001; Raulf-Heimsoth and Baur 1999, 1998; Redlich and Karol 2002; Wisnewski and Jones 2010). Alternatively, diisocyanate asthma has also been associated with IgE-independent mechanisms sharing a similar cascading pattern of activation and recruitment of key inflammatory mediators, including mast cells, eosinophils, and mononuclear cells (Raulf-Heimsoth and Baur 1998; Baur 2003; Rom 1998; Vanoirbeek et al. 2008).

Table 1-2. Percentage of IgE detection among a diverse workforce (Wisnewski and Jones 2010)

IgE+ (%)	#subjects	Patient source	Diagnosis	Isocyanate	Antigen preparation and characterization	Substitution[†]	Assay
0	11	Manufacturing Plant	Symptoms	TDI	Liquid phase, Guttman Assay	13 : 1	RAST
3	34	Various Industries	SIC*	TDI	Liquid phase, UV spectroscopy	34 : 1	RAST
14	247	Multiple Isocyanate Industries/Clinics	Physician	TDI, TMI*, MDI, MMI*	Liquid phase, derivatization w/Azo dye	26 : 1, 13 : 1, 15 : 1, 10 : 1	RAST
19	26	Cotton Seed Processing Industry	SIC	TMI	Liquid phase, UV spectroscopy	16 : 1	RAST
20	35	Finland Health Registry	SIC	TDI, MDI, HDI	–	–	RAST
21	19	Pulmonary Disease Clinics Montreal/ Quebec	SIC	TDI, MDI, HDI	Liquid phase, mass spectrometry	3 : 1 to 10 : 1	ELISA
22	23	Pulmonary Disease Clinics Montreal/ Quebec	SIC	HDI and oligomer	Vapor and liquid phases, mass spectrometry	6 : 1, 23 : 1 2 : 1	RAST
23	26	Hôpital du Sacré Coeur, Montreal, Canada	SIC	TDI, MDI, HDI	Liquid phase, chemical (TNBS)	10 : 1, 7 : 1, 24 : 1	ELISA
31	29	Hôpital du Sacré Coeur, Montreal, Canada	SIC	TDI, MDI, HDI	Liquid phase, chemical (TNBS) and immunoelectrophoresis	–	ELISA
34	58	Royal Brompton Hospital United Kingdom	SIC or Physician	TDI, MDI, HDI	–	–	RAST
38	8	Musical Instrument and Motor Vehicle Industries	Expiratory (peak) flow	TDI, MDI, HDI	Liquid phase, UV spectroscopy	–	RAST
44	66	Furniture/Musical Instrument Industries	SIC	TDI	Vapor phase, chemical (TNBS) and MALDI-MS	12 : 1	ELISA
55	11	Hôpital du Sacré Coeur & Yale Medical Clinic	SIC	HDI	Vapor phase, chemical (TNBS) and MALDI-MS	3 : 1	RAST
[†]91	12	Multiple Worsites	Physician records	TDI	Liquid phase UV spectroscopy	30 : 1	RAST

*Specific inhalation challenge (SIC).
[†]Selection criteria was physician-verified work-related immediate-type asthma attack.
[‡]Substitution = molar ratio of isocyanate to albumin (isocyanate : albumin).
–, Not reported.

Scheel et al. were credited with the first *in vitro* study to evaluate hypersensitivity reactions using TDI (Scheel, Killens, and Josephson 1964). Antibodies were generated with a reactive moiety specific to TDI when bound to human serum albumin. Karol et al. conjugated tolyl monoisocyanate to human serum albumin and used radioallergosorbent testing (RAST) to demonstrate the presence of IgE antibodies in serum of TDI sensitive workers (Karol, Ioset, and Alarie 1978). Use of monofunctional isocyanate instead of TDI, allowed for sterically exposed tolyl groups in the antigen preparation. However, the prevalence of these antibodies in workers with challenge-test-proven isocyanate-induced asthma approximated 20%. Elevated titers of tolyl-specific antibodies were not correlated with high levels of total serum IgE. This suggests

that IgE may not be the major antibody responsible for diisocyanate asthma or the antigen complex is inadequately insensitive for use as a diagnostic tool.

Bernstein et al. developed both a murine and guinea pig model to examine immune responses and sequelae using well-characterized protein conjugates (Tse, Chen, and Bernstein 1979; Bernstein et al. 1982). Briefly, the murine model showed developed homocytotropic antibodies specific to TDI conjugated with heterologous albumin with antigenicity ascribed to the ortho-tolyl side chain (Tse, Chen, and Bernstein 1979). Additionally, two different strains of parenterally immunized guinea pigs using diisocyanate-human serum albumin conjugates demonstrated heterogeneous immune responses characteristic of sensitivity reaction involving diverse immunologic and nonimmunologic effects (Chen and Bernstein 1982). Recently, Pronk et al. assessed IgE and IgG antibodies specific to hexamethylene diisocyanate (HDI) in blood samples of 581 subjects in various spray industries in the Netherlands (Pronk et al. 2007). Specific IgE was concluded to play a role in a minority of symptomatic workers while IgG indicated exposure only.

Other studies have shown that airway granulocytosis is a common acute response to MDI and TDI, and neutrolphils may be the earliest polymorphnuclear cell to mediate inflammation (Raulf et al. 1995; Hesbert et al. 1991; Fabbri et al. 1987). Neutrophil recruitment is evident by an increase in myeloperoxidase and IL-8, as well as an elevated levels of CD8+ T-cells (Fisseler-Eckhoff et al. 2011). While eosinophils are credited as effector cells of inflammation in the development of hyperresponsiveness and variable airflow limitation associated with asthma, an increase in neutrophil chemotactic activity maybe a distinguishing feature in asthmatic reactions provoked by diisocyanates (Fabbri et al. 1987; Sastre et al. 1990; Park et al. 1999). Two different studies have shown neutrophils to be present in greater amount and more frequently

than eosinophils in the sputum of subjects after challenge exposure to diisocyanates, even at low concentrations (Di Franco et al. 1998; Anees et al. 2002).

Anees et al. recruited LMW-induced asthmatic workers from the Occupational Lung Disease Unit at the Birmingham Chest Clinic to determine a cellular profile from consecutive sputum samples from worker who were still exposed at work (Anees et al. 2002). Results from this study showed that asthma caused by LMW agents can be categorized by eosinophilic and non-eosinophilic pathophysiological variants with the latter exceeding the former. While both groups had evidence of sputum neutrophilia, eosinophilic inflammation accounted for greater severity of the disease. Since a significant portion of subjects that were diagnosed with "non-eosinophilic occupational asthma" failed methacholine, this variant of asthma may be associated with a nonspecific irritant effect.

Finotto et al. compared T-cell subpopulations and circulating leukocytes after experimental inhalation of TDI (Finotto et al. 1991). Asthmatic reactions were associated with an increase in cytotoxic lymphocytes, specifically CD8+ T-cells, and eosinophils in peripheral blood. Maestrelli et al. cloned T-cells from endobronchial biopsies of asthmatics sensitized by TDI to better characterize phenotypes and profile cytokine secretions. The CD8 phenotype was also expressed in these T-cells, which produced IFN-γ, and secreted smaller amounts of IL-5, and IL-4 (Maestrelli et al. 1994).

Increased involvement of CD8+ T-cells has been suggested in diisocyanate asthma due to their axiomatic role in delayed type hypersensitivity from other low molecular weight reactive chemicals (Redlich and Karol 2002). These CD8+ T-cell results taken together with reports of CD4+ and Th1 responses, depicts a heterogenic hypersensitivity reaction to diisocyanate exposure, which at least supports a role for T lymphocytes in diisocyanate exposure.

For instance, Matheson et al. showed TNF-α to play an important role in the production of Th2 cytokines in airway tissues in response to subcutaneous injections of TDI in a murine model, independent of IgE antibody production (Matheson et al. 2001; Matheson et al. 2002).

Additional evidence supportive of T lymphocytes is diisocyanate asthma has been gleaned from peripheral blood lymphocytes responses. Bernstein et al. elucidated that the expression of a TCR Vβ repetoire, specifically Vβ1 and Vβ5, in peripheral blood mononuclear cells (PBMC) was involved in diisocyanate asthma (Bernstein et al. 1997). A skewed ratio of these two gene segments was observed in diisocyanate asthmatics, which were decreased at baseline, but selectively increased with *in vitro* incubation with diisocyanate conjugated with human serum albumin (HSA). These authors also reported findings that suggest diisocyanate-specific activation of macrophages marked by enhanced production of IL-8, TNF-a, and monocyte chemo-attractant protein-1 (MCP-1); accordingly, another possibility in the pathogenesis of diioscyanate asthma.

In vitro studies using isocyanate-albumin antigens have demonstrated activation of innate immune cells, specifically monocytes, including the production of cytokines MCP-1 and macrophage migration inhibitory factor (MIF) (Bernstein et al. 2002; Lummus et al. 1998, 1996; Wisnewski et al. 2008). MCP-1 matches histamine-releasing activity of IgE receptor cross-linking (Kuna et al. 1992).

While MCP-1 production by PBMCs has been established *in vitro*, and may substitute for IgE histamine releasing factors, cytotoxicity is a caveat in all cell-based immunoassays using isocyanates (Wisnewski and Jones 2010). Additionally, *in vitro* responses are dependent on the antigen and inclusion of appropriate antigen-presenting cells. Since most *in vitro* assays reported to date use PBMCs, key cellular profiles may be absent from peripheral blood, principally

dendritic cells, γδ T cells, and airway epithelial cell types (Wisnewski et al. 2002; Lantz et al. 2001; Lee et al. 2003).

Additionally, a progeny of adduct or conjugate formation from the reaction between MDI or TDI with self-proteins is possible under diverse conditions, and their antigenicity may widely vary (Campo et al. 2007; Ye et al. 2006). Therefore, IgE "false-negatives" are possible without accurately reflecting *in vivo* formation of the isocyanate antigen. Additionally, highly sensitive reagents are needed since IgE is present at low concentrations, and consequently missed due to detection limits of the assay, especially in serum collected after a long delay (Wide, Bennich, and Johansson 1967). Isocyanate-specific serum IgE has been shown experimentally to decrease quickly to undetectable amounts upon cessation of exposure (Tee et al. 1998).

While accuracy and reproducibility of analytical techniques and biological assays are inherently limited by an inadequate understanding of the complexity of pathophysiological mechanisms underlying diisocyanate –induced asthma, certain host factors certainly mediate susceptibility or resistance to sensitization in exposed workers given the IgE-response rate previously reported (Raulf-Heimsoth and Baur 1998; Redlich and Karol 2002). Human leukocyte antigen (HLA) class II alleles are associated with antigen presentation to CD4+ T-cells in relation to Th2-driven/IgE response (Choi et al. 2009; Mapp et al. 2000; Fabbri et al. 1995; Bignon et al. 1994; Kim et al. 2006). While HLA class II genes are most likely located in the promoter region of numerous allergy responsive genes, Bignon et al. found that the DQB1*0503 allele along with the allele combination DQB1*0201/0301 were linked to susceptibility to the disease (Bignon et al. 1994). The alleles and haplotype DQB1*0501 and DQA1*0101/DQB1*0501/DR1, respectively, were protective. These findings were substantiated by Mapp et al. (Mapp et al. 2000). Additionally, Choi et al. enrolled 258

individuals in their study to investigate HLAI and HLAII polymorphisms, 84 of the patients showed diisocyanate-induced asthma (Choi et al. 2009). The haplotype HLA DRB1*1501-DQB1*0602-DPB1*0501 and allele DQB*0402 were found more frequently in TDI-induced patients and IgE antibodies specific to TDI-albumin conjugates.

Piirila et al. screened 182 diisocyanate workers, including MDI and TDI, for polymorphisms in four glutathione *S*-transferase genotypes, specifically GSTM1, GSTM3, GSTP1, and GSTT1 (Piirila et al. 2001). Patients with the GSTM1 null genotype were at a 1.89-fold greater risk to develop isocyanate-asthma consistent with latent reactions. These patients rarely produced IgE antibodies specific to isocyanate. The variant Val^{105}/Val^{105} GSTP1 genotype was found with a frequency of 9.2% in the patient group, and associated with elevated levels of IgE antibodies. However, this finding is incongruent with the results in a similar study that found Val^{105}/Val^{105} GSTP1 was protective evident by a decreased risk for isocyanate-induced asthma (Mapp et al. 2002). Differences in isocyanate exposure, definitions of asthma, bronchial hyperresponsiveness, exposure level and duration, and small allele frequency in the cohorts may account for the differences. Nevertheless, polymorphic GSTs may be an important determinant of risk and type of immunologically mediated diisocyanate-induced asthma.

Diisocyanate asthma diagnosis, screening, and surveillance remain a challenge without a defined presence and role of allergen-specific IgE (Wisnewski and Jones 2010). Bronchial asthma is the first clinical diagnosis in symptomatic diisocyanate-exposed workers, and by then lung function decline may have long-lasting effects (Raulf-Heimsoth and Baur 1998; Wisnewski and Jones 2010). Furthermore, without a complete and detailed occupational history, isocyanates may be overlooked as the cause of disease in workers who leave the job-site, especially in delayed responses (Wisnewski and Jones 2010; Bardana, Montanaro, and

O'Hollaren 1992). Instead, asthma may be attributed to environmental triggers, leaving the worker/patient without disability or compensation.

The number of acute and chronically exposed workers will continue to grow with the popularity of PUR products. Therefore, accurate measurements are essential in preventing potential worker exposures to these pervasive airway sensitizers.

Hypersensitivity Pneumonitis

Hypersenstivity Pneumonitis (HP), also known as extrinsic alveolitis, is an immunologic syndrome that differentiates a spectrum of lung diseases, including granulomatous, interstitial, and alveolar-filling, caused by repeated inhalation of and sensitization to a wide variety of etiologic agents, specifically MDI and TDI (Schwarz and King 1993; Grammer 1999; Tripathi and Grammer 2001). Three generally recognized syndromes related to HP are: acute, subacute, and chronic (Grammer 1999). The hallmark of the acute form of HP is T lymphocytes in bronchoalveolar lavage (BAL), which are mostly activated CD8+ T cells, concomitant with mononuclear cell infiltration in the interstitium. A CD4/CD8-ratio <1.0 in BALF analysis is a good predictor of lymphocytic alveolitis(Baur 1995). Other lines of HP evidence in BAL fluid and peripheral blood are the involvement of IgG and cellular components of Gell and Coombs type III and type IV immunity (Raulf-Heimsoth and Baur 1998) Histological finding of fibrotic tissue (mild interstitial fibrosis) distinguishes chronic form of the disease from the acute forms. Granulomatous pneumonitis is the first clinical indication of the illness, and is usually reversible upon cessation of exposure and administration of oral steroids or other immunosuppressive agents (Schwarz and King 1993). Continued, repeated exposure may culminate into chronic fibrosis mediated by sensitization and attendant immunological damage to the lung.

Most reported cases of HP following exposure to MDI or TDI, most commonly MDI, have an acute or subacute presentation with nonspecific symptoms of dyspnea, fever, chills, which may go unrecognized or be misdiagnosed without a high degree of clinical suspicion (Rom and Markowitz 2007). The data available are focused chiefly on farmers' lung disease, which has made prevalence of HP in isocyanate workers quite challenging. Limited cross-sectional investigations following index cases of isocyanate-induced HP has produced a wide variance of prevalence. In one study of 167 workers exposed to resin based MDI used in the manufacture of woodchip boards found at least eight confirmed cases of HP (~5%) based on positive specific challenges to MDI and presence of MDI-IgG and MDI-IgE antibodies. An augmentation of prevalence may have occurred since only nine symptomatic workers underwent evaluation.

Respiratory and/or systemic symptoms were reported in twenty-three of 34 foam injection workers at an automobile manufacturing plant (Simpson et al. 1996). The overall prevalence of HP was approximately 30% among line operators in the injection molding process. However, confirmatory studies were not performed.

Baur concluded occupational exposure to isocyanate vapors and aerosols are capable of inducing HP in at least 1% of a group of 1780 isocyanate workers with symptoms (Baur 1995). In a more recent study, Schrieber et al. report a case of occupational HP as a result of low-level exposure to diisocyanates (Schreiber et al. 2008). Briefly, a secretary of a car body repair shop developed nonspecific symptoms (e.g.,, cough and fatigue) after a year of regular employment. Two years later, a chest radiograph revealed bilateral ill-defined parenchymal opacities and computed tomography scan showed reticulonodular and discrete ground-glass pattern. IgG antibodies specific to diisocyanate human serum albumin conjugates were detected up to

approximately four-fold elevation. The company used hardeners and adhesives that contained HDI and MDI, respectively. Monomer concentrations were determined according to German standard procedures for the detection of airborne diisocyanates.

RADS

Reactive airways dysfunction syndrome (RADS) is an asthma-like illness without latency, developing after a single high-level exposure to MDI or TDI (Redlich and Karol 2002; Leroyer et al. 1998). Non-immunological mechanisms likely account for RADS, and the effects may last for months to years (Redlich and Karol 2002). Leroyer et al. described a case history of a foundry worker that presented with RADS following an exposure to MDI. Upon the patient's return to work, re-exposure to low concentrations designated as nonirritant occurred. Persistent symptoms were reported and a baseline FEV1/FVC was 2.8/4.3 and methacholine challenge confirmed hyperresponsiveness six-months after the acute inhalation. This case report suggests sensitization that transformed RADS into occupational asthma from isocyanate exposure.

Biomarkers

Biological monitoring is the measurement of exposure determinants, called biomarkers, which can be: the original exposing chemical, a metabolite, or a reversible biochemical change (e.g.,, conjugates, adducts, or enzyme modification) (DiNardi and American Industrial Hygiene Association. 2003; Hygienists 2012). These determinants can be measured in blood, urine, exhaled air, and other biological specimens (e.g.,, hair, nails, breast milk, sputum). Currently, there are only three biological monitoring criteria mandated by the U.S. federal government, which are concerned with exposure to: HIV in first responders and medical waste clean-up; as well as, two toxic metals, lead and cadmium.

At present, neither ACGIH nor NIOSH have recommended methods for biomonitoring MDI or TDI. The ACGIH biological exposure indices (BEIs) committee has proposed a notice of intended changes (NIC) for TDI-2,4-, or 2,6- or a mixture of the isomers (Hygienists 2012). The "Deutsche Forschungsgemeinschaft" Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has established a list of maximum workplace concentrations (MAK), and biological tolerance value for occupational exposure (BAT) values, which in 2007 exceeded more than 90 substances considered to be xenobiotics (Drexler, Goen, and Schaller 2008). Presently, this German Commission is the only agency to recommend a biological exposure limit for diisocyanates, specifically for MDI based on a BAT value of 10μg/g creatinine of 4,4'-methylenedianiline (MDA) in urine (van Kampen, Merget, and Baur 2000; Sennbro et al. 2005).

BEIs are a collection of published reference values that complement exposure assessment by air sampling so that nearly all workers can be repeatedly exposed to without adverse health effects (DiNardi and American Industrial Hygiene Association. 2003; Hygienists 2012). BEIs require metabolic and pharmokinetic data, which enhances the utility of BEIs as an indicator of the uptake of a substance; accordingly, BEIs are the best tools available for determining body burden of chemicals. BEIs are to be understood as averages, as opposed to ceiling values that do not allow intra-individual variability that may permit value transgression in the individual employee (Drexler, Goen, and Schaller 2008). While an NIC has been proposed for TDI and there is surmounting evidence demonstrating the non-health based utility of isocyanate biomonitoring to assess efficacy of controls, no reliable method of serological testing has been established to date, which also differentiates between markers of exposure and markers of disease (Palikhe, Kim, and Park 2011; Cocker 2011; Brown and Burkert 2002).

Due to the complex pathogenic mechanisms of isocyanate-induced asthma, potential biomarkers have been proposed based on the direct and indirect effects of MDI and TDI. For instance, the analysis of pre- and post-hydrolysis of fluids and molecules to liberate TDA was one of the earliest techniques used to determine TDI exposure. In 2005, Sennbro et al. calculated an upper reference limit (URL) for TDA and MDA, biomarkers for TDI and MDI, respectively, in hydrolyzed urine and plasma from 121 occupationally unexposed workers (Sennbro et al. 2005). These workers exhibited detectable biomarker levels of MDI and TDI, especially abundant was MDA found in 97% of both urinary and plasma samples analyzed. URLs ranged from 0.1-0.4 mg/L and 0.4-0.5mg/L of plasma or urine for TDA (2,4-, and 2,6 isomers), and MDA, respectively. These values may be used in screening for occupational exposure, which is deemed positive with a biomarker level greater than the respective URL. However, hydrolysis analyses assume that biological macromolecules are completely hydrolyzed under the conditions utilized to which there are a range of procedures, internal standards, extraction methods, derivatives, and detection (Brown and Burkert 2002; Cocker 2011).

A number of other biomarkers have been identified as a result of chemical modification of particular molecules, including conjugates with hemoglobin, glutathione, laminin, tubulin, and serum albumin (Brown and Burkert 2002). These conjugates most likely drive diisocyanate antigenicity through neoepitope formation, especially since diisocyanates have a molecular weight less than 1000 Da (Rom 1998; Ye et al. 2006; Wisnewski, Liu, and Redlich 2010). Cell- and/or antibody-mediated (innate and adaptive immunity, respectively) immune responses to isocyanates may elicit a variety of illnesses and disorders (Raulf-Heimsoth and Baur 1998). However, while isocyanates may act as haptens, only one self protein is known to generate an *in vivo* human humoral response by creating a pathogenic antigen on reactivity with isocyanate,

which is albumin (Wisnewski, Liu, and Redlich 2010). Therefore, investigations have predominantly focused on cellular responses, specifically lymphocyte transformation and mixed lymphocyte reactions using human-serum albumin (HSA) (Rom 1998).

Kumar et al. presented the first work to quantify isocyanate-specific adducts with albumin in rats using LC-MS/MS (Kumar, Dongari, and Sabbioni 2009). Albumin was isolated from chronically exposed rats for three months to three different doses of MDI aerosols. Synthesis of isotope labeled compounds as internal standards, which corresponded to the adducts found in vivo, allowed for both identification and quantitation of an isocyanate-specific albumin adduct. MDI formed an adduct with N_e-lysine of albumin, and increased linearly from 0-24.8pmol/mg albumin.

In a follow-up study, Kumar et al. collected plasma and urine from two different worker groups (i.e., chemical industry and construction) with known exposure to MDI and prepolymer products to assess their new biomonitoring procedure (Sabbioni, Dongari, and Kumar 2010). MDI was again shown to react with the available epsilon amino group of lysine, forming the MDI-albumin adduct. MDI-Lysine levels were significantly higher in the exposed workers vs. control workers (Mann-Whitney test, $p<0.01$). The 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile of MDI-Lysine levels for construction and factory workers were 0, 65.2, 134, 244 fmol/mg and 0, 30.5, 57.4, 95.8 fmol/mg, respectively.

Several groups have detected serum specific IgE antibodies to both MDI and TDI-HSA conjugates in the sera of workers with a positive bronchial challenge (Baur, Dewair, and Fruhmann 1984; Tee et al. 1998; Sabbioni, Dongari, and Kumar 2010; Palikhe, Kim, and Park 2011). However, the prevalence varied considerably depending on conjugate preparation and

characterization, and selection of immunoassay methods to detect antibody (Palikhe, Kim, and Park 2011; Ott et al. 2007).

Technical issues surround conjugate preparation and characterization, mainly because of a lack of standardization across laboratories (Ott et al. 2007). For instance, methodological variables that must be considered are choices involving: specific diisocyanate formulation to be conjugated; carrier protein; reaction conditions; and, post-reaction processing for preparation; as well as, Gutman assay, mass spectrometry, or electrophoresis for characterization.

Ye et al. generated TDI-albumin conjugates using both liquid and vapor phases of TDI to determine antigenicity dependence on the biophysics of exposure (Ye et al. 2006). The physical state of TDI (vapor vs. liquid) demonstrated measureable differences based on migration in native gels, and matrix-assisted laser desorption/ionization-mass spectrometry mass/charge spectra. IgE from 44% of subjects with TDI asthma recognized the vapor TDI-albumin conjugates compared to a 17% response rate to liquid TDI-albumin conjugates in these same patients. Putatively, serology effectively identifies TDI asthmatics and exposed workers if the appropriate form is prepared for analysis.

Immunoassay methods for determining antigenicity of diisocyanate-protein conjugates have included ^{125}I-labeled IgE or IgG or enzyme-labeled anti-human IgE or IgG in both RAST and enzyme-linked immunsorbent assay (ELISA), respectively (Ott et al. 2007). Intra- and inter-variability between these assays may originate from non-standardized protocols for antigen binding variable, antigen attachment methods, serum incubation period, which together with differences in conjugate processing can impact the test performance and account for the inability to reliably compare results between groups (Ott et al. 2007; Brown and Burkert 2002).

Correlating a binary interpretation of uptake and adverse effect requires establishing criteria to define and interpret test outcomes in order to prevent misapprehensions (Brown and Burkert 2002; Ott et al. 2007). However, differences in serum dilution levels, confirmation of positive results, cross-reactivity, and study design may bias test performances and interpretations. Additionally, health events consistent with TDI exposure are a series of changes that have the potential to release different markers at different onset points and potential effect starting points (Figure 1-30) (Brown and Burkert 2002).

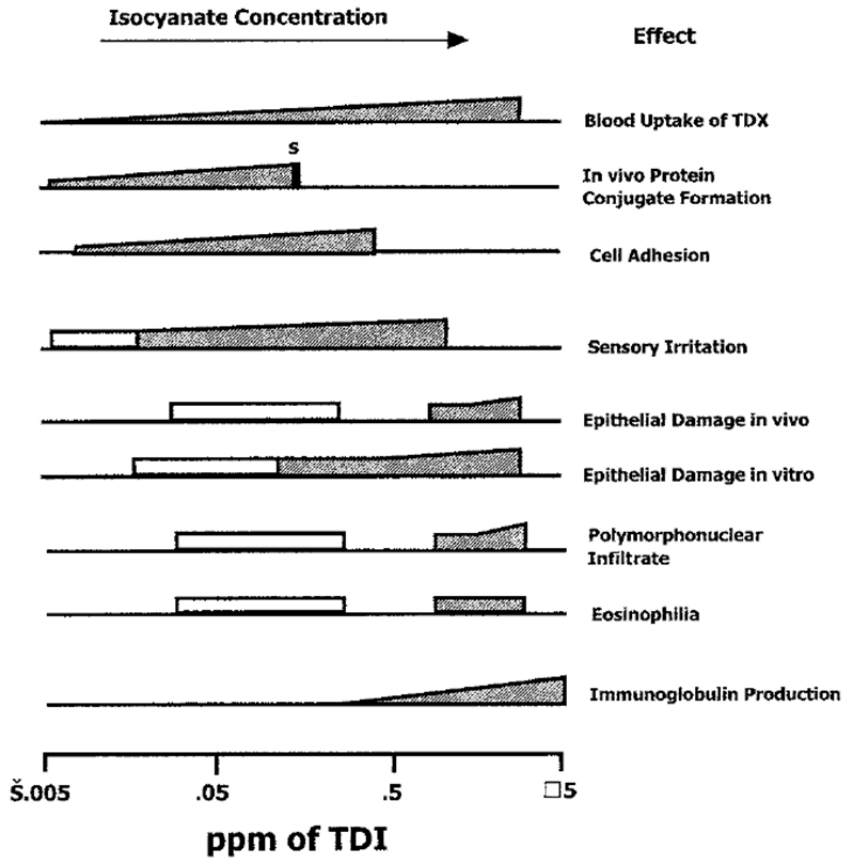

FIGURE 1-30. Concentration-specific effects of TDI exposure leading to different biomarkers at different onset points (Brown and Burkert 2002).

Wisnewski et al. developed a murine model to discern a putative role of albumin in respiratory responses to skin exposure to MDI (Wisnewski et al. 2011). Various concentrations of MDI were formulate in acetone/olive oil and applied to the skin. Mouse sera samples were

analyzed for MDI-specific antibodies, specifically IgE and IgG, using ELISA with microtiter plates coated with murine MDI-albumin conjugates. This group found that the end titers associated with MDI concentrations of 0.1-10% weight/volume exceeded 1:100,000 and 1:30,000 for IgG1 and IgG_{2a} subclasses, respectively. Concomitantly, MDI-specific IgE and total IgE serum levels were elevated six times that of control levels. This study confirmed albumin as a major target of MDI conjugation capable of eliciting a humoral response. Furthermore, the MDI-specific IgG ELISA assay may be an important tool in detecting workplace exposure.

Isocyanate-specific IgE and IgG antibodies have been studied since the 1970s for use as a biological marker of diisoycanate-induced asthma (Brown and Burkert 2002). While their presence facilitates diagnosis, their presence alone is not sensitive enough as a biomarker, and presents knowledge of exposure only.

Ott et al. reviewed 29 articles in current literature of 2007, which met specific inclusion criteria, to assess prevalence estimates of specific IgE and IgG binding to diisocyanate conjugates by disease and exposure status (Ott et al. 2007). IgG-specific responses to dissocyanate conjugates were found to be more prevalent in both occupational and non-occupational cohort (e.g.,, residents living near a flexible foam production facility) (Ott et al. 2007; Palikhe, Kim, and Park 2011; Orloff et al. 1998; Littorin et al. 2000; Skarping et al. 1996; Redlich et al. 2001). However, positive IgG responses were also noted in healthy employees without diisocyanate asthma, clouding its role as a reliable indicator of disease status (Baur 1995). Another disconcerting element in the usage of IgG antigenicity as a specific diagnostic tool for diisocyanate asthma is the relative risk of a positive test among known cases versus non-

cases was less for specific IgG than for specific IgE across all conugates and in both stratified and non-stratified analyses (Ott et al. 2007)

Serum autoantibodies to increased cytokerratin expression or tissue transglutaminase were also elevated in exposed groups with TDI-induced asthma compared to asymptomatic exposed controls, however the prevalence and sensitivity of these candidate biomarkers were not high enough (Palikhe, Kim, and Park 2011). Protein markers (e.g.,, serum ferritin, transferring, and serum vitamin D binding protein) and serum cytokines (e.g.,, myeloperoxidase, interleukin-8, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, and VEGF) have also been postulated, and studies are ongoing. Currently, matrix metalloproteinase-9 is showing enhanced applicability as a serologic marker for predicting TDI-inducded asthma, especially considering the cost-benefit ratio (Palikhe, Kim, and Park 2011).

As previously reported (see Asthma section), genetic biomarkers may also serve as potential candidates since factors such as allele, genotype, or haplotype frequencies may increase susceptibility to diisocyanate-induced asthma.

Biological monitoring and BEIs serve as an ancillary dimension in understanding exposure and disease through ADME generated information and indirectly facilitates medical screening and surveillance efforts in the diagnosis and prevention of disease. Biomarkers/biological monitoring should not replace traditional industrial hygiene air sampling, but rather used as a complement in exposure assessment (Hygienists 2012).

Occupational Exposure Limits and Sampling Methods

Workers may encounter chemical and physical agents that may have chronic effects without indication from short-term exposures (Allport 2003). Occupational exposure limits (OEL) to chemical and physical agents are provided to ensure safe working conditions over the

working lifetime of an individual. Since inhalation is the primary route of exposure in the workplace setting, many groups have established occupational exposure limits for airborne species. These OELs are calculated by regulators, academic bodies, or industry from pooled resources including experimental animal studies, human data from clinical cases and epidemiological studies, chemical structure-biological activity models, and chemical-specific toxicology data (Allport 2003; DiNardi and American Industrial Hygiene Association. 2003).

While many of these aforementioned groups within the United States recommend OELs for workplace health professionals, the OSHA and Environmental Protection Agency (EPA) are the only regulating authorities capable of enforcing promulgated maximum workplace limits through legally binding permissible exposure limits (PELs) and new chemical exposure limits, respectively (DiNardi and American Industrial Hygiene Association. 2003; Allport 2003). The NIOSH publishes criteria documents for recommended exposure limits (RELs), which may be used in setting PELs. Additionally, the ACGIH also publishes threshold limit values (TLVs) that are routinely re-evaluated and adapted accordingly to newly available data (Allport 2003). While ACGIH TLVs are not legally enforceable, these guideline values present workplace limits that are typically more contemporary and conservative than the OSHA PELs.

Approximately 400 OSHA PELs became mandatory in 1970 under the Occupational Safety and Health (OSH) Act, which were adopted from a 1968 list of TLVs and the standards of the American National Standards Institute (ANSI) (DiNardi and American Industrial Hygiene Association. 2003). Since that time, only 12 limits have been modified or added. In July 1992, the 11th Circuit Court of Appeals overturned the 1989 OSHA PEL Air Contaminants Standard (Streicher 1994). Consequently, OSHA enforces 1971 transitional values codified in Table Z-1-A in the Code of Federal Regulations.

Accordingly, the PELs for airborne MDI and TDI are based on an antiquated TLV list, which prescribes exposure criteria for MDI and TDI monomers only using a ceiling limit of 20 ppb, respectively (Bello et al. 2004; Administration). ACGIH defines the ceiling limit as the exposure that should not be exceeded during any part of the working exposure (Hygienists 2012). There are no PELs or TLVs for the higher molecular weight oligomers or polyisocyanates of MDI and TDI (Bello et al. 2004; Booth et al. 2009)

OSHA does not mandate a TWA standard for MDI or TDI, but the protocol for monitoring is to assess the ceiling over a 15-minute period (Bello et al. 2004). Consequently, instantaneous concentrations may exceed the ceiling limit, while the average may very well be within the legal requirements of 20 ppb for airborne aromatic diisocyanates. This underestimation is an important caveat in regulatory compliance and sampling strategy for both MDI and TDI since peak exposures may be more relevant than lower long-term, or cumulative exposures in terms of risk for developing symptoms of asthma (Allport 2003; Mapp et al. 1988; Mapp et al. 2005; Chan-Yeung and Malo 1995).

The ACGIH TLV and NIOSH REL issue both a daily time-weighted-average (TWA) for an eight-, or 10-hour day, respectively, and a 10-minute ceiling and 15-minute short-term exposure limit (STEL), respectively, as illustrated in Table 1-3. (Corporation 1997) The United Kingdom Health and Safety Executive (UK-HSE) exposure criteria for isocyanate species is also included in this table signifying an alternative metric, which is based on total number of reactive isocyanate groups (TRIG) in a volume of air (Streicher 1994). TRIG, as an exposure metric, is a comprehensive approach to monitoring isocyanate, which circumvents possible methodological limitations in monitoring exposures to single isocyanate species. Briefly, depending on the industry and product, mixtures of isocyanates (e.g., monomers and/or polyisocyanates) may be a

more accurate reflection of an isocyanate exposure. Also of note in this table is the 1989 recommendation of NIOSH, which considers TDI to be an occupational carcinogen that should be limited to the "lowest feasible limit".

TABLE 1-3. NIOSH and ACGIH short term and ceiling limitsexposure criteria for aromatic and aliphatic isocyanates (Streicher 1994; Streicher et al. 2000).

Isocyanate Species	*Exposure Criteria - Full-shift TWAs Micrograms per cubic meter of air*			*Exposure Criteria - Short Term or Ceiling Limits Micrograms per cubic meter of air*			
	NIOSH REL	ACGIH TLV	UK-HSE	NIOSH REL Ceiling	ACGIH TLV-STEL	UK-HSE Ceiling	OSHA PEL Ceiling
TDI	CA-LFC[1]	36	None	None	140	None	140
MDI	50	51	None	200	None	None	200
HDI	35	34	None	140	None	None	None
HMDI	None	54	None	210	None	None	None
IPDI	45	45	None	180	None	None	None
NDI	40	None	None	170	None	None	None
TRIG[2]	None	None	20	None	None	70	None

[1] NIOSH considers TDI to be an occupational carcinogen (CA) and recommends that exposures be reduced to the lowest feasible concentration (LFC).
[2] TRIG - total reactive isocyanate group.

The findings of Hama and Elkins et al. established the first TLV-ceiling of 0.02 ppm for TDI, which was established from 1963-1982 (Hama 1947; Elkins 1965). Subsequently, the TDI TLV has evolved to its most recent recommendation of a TLV-TWA of 0.005 ppm (0.036 mg/m^3), with a 15-minute STEL of 0.02 ppm (0.14mg/m^3) for TDI mixtures and isomers. Additionally, notations SEN and A4 have been assigned to designate allergic sensitization and Not Classifiable as a Human Carcinogen, respectively. The A4 assignment was based on equivocal evidence from the U.S. National Toxicology Program and a 1983 study by Loeser (Loeser 1983; Program 1986).

The MDI TLV has tracked TDI's TLV-TWA chronology such that an analogous value of 0.005 ppm (0.051mg/m^3) has been recommended for present day occupational exposures to MDI due to toxicological similarities with TDI, but limited also by definitive exposure data (Hygienists 2001). Such a limitation has prohibited Skin, SEN, or carcinogenicity notations, as

well as a TLV-STEL for MDI, as seen for TDI. Therefore, the present MDI TLV-TWA while minimizing potential for both pulmonary function decrement and respiratory tract sensitization, may not protect those workers susceptible to sensitization, or sensitized workers from allergic reactions.

The current TLV documentation, which supports the derivation of the present MDI and TDI TLVs, is a compilation of fundamental studies that have documented health effects from both animal and human data in a defined range of exposure concentrations (ACGIH). A collection of these studies focused on the causal relationship between exposure levels and sensitization, as well as decrement in health of workers after removal from exposures. Both MDI and TDI documentations cite a gamut of animal and human studies to justify their TLV recommendation, but in view of MDI sharing the same TLV-TWA as TDI—a few of the key studies used to recommend only the current TDI TLVs will be presented below.

Early investigations of chronic low-level exposures (e.g.,, below 0.02 ppm) demonstrated the onset of sensitization to an adhesive containing TDI (Markham and Fishburn 1967). Additionally, results from a compilation of studies conducted by Wegman et al. revealed a dose-response relationship between exposure levels and a decreased FEV_1, as much as 206 ml in two years (Wegman 1974; Wegman et al. 1982; Wegman et al. 1977). This pulmonary function loss exceeds the expected value by three- to fourfold even at 0.002 ppm, indicating that workers, especially sensitized, may not be protected at this exposure level.

Peters et al. examined worker exposure to TDI below the 0.02 ppm TLV-ceiling during polyurethane foam production (Peters et al. 1968; Peters, Murphy, and Ferris 1969). A profile of airborne TDI was determined in highly populated work areas of the plant, as well as pulmonary function measurements of 34 workers on Monday morning, afternoon, and Friday afternoon.

Significant differences were observed in worker FEV_1 measurements comparing these three measurements. Greater declines were also noted in symptomatic versus asymptomatic workers. These acute changes lead this group to conclude that even a TLV of 0.01 ppm is too high.

Butcher et al. identified clinically sensitized workers from a study population at a TDI-producing plant and challenged them with 0.005 ppm TDI vapor (Butcher et al. 1976). Classic immunologic responses were elicited in these workers: immediate, sustained late reaction, and dual immediate and delayed reaction. Environmental exposures to less than 0.001 ppm TDI were demonstrated in the etiology of asthma, including do-it-yourself PUR products (Peters and Murphy 1971; Carroll, Secombe, and Pepys 1976).

Symptoms of diisocyanate exposure, including mucopurulent bronchitis, were reported to persist 40 months to 17 years after cessation of exposure in some workers manufacturing TDI, and handling polyurethane resins (Adams 1970, 1975; Innocenti, Franzinelli, and Sartorelli 1981). These studies have also protracted the establishment of a no-effect for both MDI and TDI. Therefore, conservative exposure limits, particularly the TLV (e.g.,, 0.005 ppm) , should be considered when implementing controls and intervention compared to the OSHA PEL (0.02 ppm). Otherwise, susceptible workers may inadvertently experience either sensitization or allergic reactions (Hygienists 2001).

Sampling

A variety of sampling techniques and analytical methods exist for MDI and TDI measurements in multimedia distributions and partitions in environmental compartments (Allport 2003). For instance, a collection of methods has evolved for monitoring MDI and TDI in workplace atmospheres from poured or sprayed applications that generate vapors and/or aerosols. Nonairborne analysis of MDI and TDI include heterogeneous test methods for

successful characterization of diisocyanate contamination and decontamination of water and soil from accidental releases and clean-up operations, respectively. Additionally, quality assurance methods for bulk diisocyanates and polyurethane products (e.g.,, offgasing or thermal degradation) have also been established (Allport 2003).

Aerosol exposures are predominantly encountered in the workplace during the use of MDI or TDI; especially, in the auto body industry where painter and technicians spray PUR paints for refinishing work (e.g.,, clearcoats) (Redlich and Karol 2002; Reeb-Whitaker et al. 2012; Pronk et al. 2006). Accordingly, these end-users of isocyanates are at the greatest risk of developing isocyanate asthma even if higher molecular weight forms, which do not readily volatilize, replace their respective monomers due to either heating or mechanical aerosolization of the isocyanate compounds (Pronk et al. 2006; Liu et al. 2007; Di Stefano 2004; Streicher et al. 2000).

Therefore, accurate measurements of MDI and TDI in workplace atmospheres are crucial, especially for previously sensitized workers. Quantitative determinations rely on capturing a representative sample, which puts the onus on both the occupational health practitioner and the sampling method. Isocyanates, characteristically, have an extremely varying disposition in the workplace that make sampling of airborne MDI and TDI quite difficult, especially in spray applications (Pronk et al. 2006; Redlich and Karol 2002; Rom and Markowitz 2007; Streicher et al. 2000; Allport 2003; Bello et al. 2004). Episodic exposure profiles, high reactivity and absorptivity to particulate matter, and possible presence of either (or mixture of) monomeric vapor, and aerosols of varying particle sizes along with the presence of many other different chemicals (e.g.,, polymeric species and interfering additives contained in the polyol mix) account for the technical difficulty in quantitative determinations especially at or below

exposure limits (Allport 2003; Streicher et al. 2000; Redlich and Karol 2002; Lesage et al. 2007; Streicher 1994). Other sources of limitations and errors that may promote isocyanate losses and underestimations, include: sample preparation, storage, shipping; and, analyte separation, identification, and quantification (Allport 2003; Streicher 1994).

Despite this complex matrix of varying vapor pressures, solubilities, and particle sizes potentially in the workplace air, all major isocyanate species (e.g.,, monomeric, prepolymeric, polymeric) are capable of inducing asthma in exposed workers (Redlich and Karol 2002). However, deposition in various regions of the respiratory system may occur due to different sized inhalable fractions of isocyanate particles, which, depending on the size, are governed mostly by impaction, settling, and diffusion mechanisms (Hinds 1999). As a result, different physiological responses are likely to occur based on exposure duration, potency, dose, and concentration of deposition in the respiratory tract (Hinds 1999; Streicher et al. 2000).

Pauluhn et al. described induction and sensitization of the respiratory system in guinea pigs from inhalability of isocyanate aerosols of two different particle sizes (Pauluhn et al. 2000). A greater acute response based on increased respiratory rate was determined when the mean mass aerodynamic diameter (MMAD) was less than 2μm rather than approximately 5μm. Topographical locations in the lung were examined for influx of eosinophilic granulocytes, which showed greater recruitment of eosinophils per unit area in the bronchial perivascular and lung-associated lymph nodes (LALN) when the animals were exposed to the larger aerosol. A higher rate of sensitization was implicated in exposures to the larger aerosols due to a greater fraction of deposition in the upper respiratory tract.

Depending on the application of the isocyanate, condensation aerosols may form at less than 0.1μm when heated just below thermal degradation temperature (Inspection 2001).

Conversely, particle sizes greater than 20μm may be generated, in addition to gaseous isocyanates (Ackley 1980; Bosseau 1992). Lesage et al. used Marple 8-stage impactors to obtain particle size distribution of airborne MDI associated with PUR spray foam in residential construction (Lesage et al. 2007). A mean respirable fraction of 20% was determined with a majority of the particles greater than 10μm during the spray foam aerosol generation. Additionally, several studies report varying aerodynamic diameters during spraying of PUR paint, specifically greater than 2μm or 20μm (Inspection 2001; Dahlin et al. 2008).

Large variability in overspray particle size distributions generated during manual spray painting using compressed air has been attributed to several different task-based parameters, which include spray nozzle pressure, air-to-liquid mass flow ratio (Carlton 1997). Additionally, Carlton and Flynn determined that worker orientation to the spray booth free stream influences the size distribution in closed-face cassette sampling in both conventional and high volume, low pressure (HVLP) air spraying (Carlton 1997). Statistically significant differences were seen in geometric mean diameters for both 90° and 180° worker orientations. In both field and laboratory collected samples, the 180° orientation showed a higher mass percentage in the smaller sizes but less mass in the larger sizes. The 90° orientation where the worker stands with the freestream to the side experienced larger size distributions versus the 180° worker orientation. Overspray transport mechanisms most likely explain these differences.

Ideally, a sampler must collect both gas and particles in a representative way, which is also capable of separating the analytes into separate size fractions (Dahlin et al. 2008; Streicher et al. 2000; Henneken, Vogel, and Karst 2007; Marand et al. 2005; Huynh 2009). However, convenience, simplicity, and speed of sampling and analysis have to be considered when

choosing each methodology, which will ultimately impact collection efficiencies of MDI and TDI.

Other considerations are collection errors in the sampling method, which impact the capture efficiency of particulate isocyanates (Streicher et al. 2000; Tucker 2007; Allport 2003). Collection errors can be attributed to aspiration, internal wall, and transmission losses. Aspiration errors may introduce a positive or negative bias in the results and not accurately reflect human inhalation efficiency. This error occurs when the probe intake stream does not match the velocity of the air stream.

Internal wall losses account for deposition of particles along the internal components of the sampler that are not available for subsequent desorption/wash and analysis. Transmission losses can occur from breakthrough or complete penetration of particles through the sampler.

To assess worker exposure, air may be drawn into a direct reading device (e.g.,, paper tape system) or through an integrated sampling device, which captures isocyanates by absorption into a liquid or adsorption onto a filter or glass wool (Allport 2003; DiNardi and American Industrial Hygiene Association. 2003). Typically, these collection methods utilize a chemically impregnated media to stabilize the isocyanate, and improve analytical detection (Streicher 1994). Filters and paper tapes are prepared with a derivatizing agent capable of being quantitated by compatible methods, which include colorimetric, UV, fluorescence, electrochemical detection, mass spectroscopy or other spectroscopic methods (Allport 2003).

Derivatizing agents are typically primary or secondary aliphatic amines, which have shown the best results for isocyanate analysis through better stability and molar absorptivity (Streicher et al. 2000; Henneken, Vogel, and Karst 2007). Common derivatizing agents include dibutyl amine, N-4-nitrobenzyl-N-*n*-propylamine (nitro reagent), 3-(2-aminoethyl)indole

(tryptamine), 1-(2-methoxyphenyl) piperazine (MOPP), 1-(2-pyridyl) piperzine (1-2PP), 9-(methylaminomethyl) anthracene (MAMA), 1-(9-anthracenylmethyl) piperazine (MAP) (Streicher et al. 1996).

All these amine-based reagents are nucleophilic compounds with a penchant for reacting with the electrophilic diisocyanate functional groups with only slight differences in the reactivity (Henneken, Vogel, and Karst 2007; Streicher et al. 2000). However, accessibility of the reagent to isocyanates is a rate-limiting step (Streicher et al. 2000). For instance, efficiency of mixing is related to isocyanate segregation from aerosol mixtures, which may preclude needed contact between the reagent and analyte (Streicher et al. 1996; Streicher et al. 2000).

While the reactivity between these reagents has been suggested as nominal, novel reagents and media, and state-of-the-art analysis are frequently introduced as the gap narrows in understanding isocyanate sampling and monitoring. For instance, certain reagents have been developed with lower limits of detection, limits of quantitation, and low compound-to-compound variability. Therefore, downstream methods in the quantitative strategy, specifically detection procedures, are heavily influenced by the choice of derivatizing agent and its capabilities.

Furthermore, inherent stability and high molar absorptivity of certain derivatizing agents may provide unmatched limits of detection when coupled with appropriate instrumentation. A variety of other derivatizing agents have since been developed, most recently 4-methoxy-6-(4-methoxy-1-napthyl)-1,3,5-triazine-2-(1-piperazine) (MMNTP). This new derivatizing agent has exhibited promising characteristics, specifically small compound-to-compound variability between aromatic and aliphatic diisocyanates (Werlich 2004).

Such endeavors have lead to the ACGIH TLV intended changes for TDI. ACGIH ratifies proposed TLVs as notice of intended changes for a calendar year before final adoption or

withdrawal, which champions that exposure limits should not be set based on methodological limitations. Rather, ratification of occupational exposure limits must be based on contemporary, causally related exposure health effects data with methodology vetted for sensitivity and accuracy that satisfy the requirements of ACGIH TLV for TDI and MDI. For example, 1-2PP, which is still used by OSHA, may not be as good of a standard for airborne isocyanates as compared to MAP in light of new studies that show total reactive isocyanate groups (TRIG) may be an appropriate exposure metric. The NIOSH draft methd 5525, which is based on MAP derivatization, is being considered by the International Organization for Standardization working group (ISO/TC 146/SC 2/WG 4) as an ISO Norm for the measurement of workplace atmospheres, which cover airborne mono-, di- and poly-isocyanates.

Direct-reading instruments are most commonly based on the paper tape technology (Allport 2003). The paper tape system produces a color stain that is measured photometrically (Allport 2003). The intensity of the color is proportional to the concentration of isocyanates in the air. Some examples include GMD Sure Spot ^{TM} equipment, TDI vapor detection badges, ion-mobility spectrometry-based TDI monitors, and colorimetric tubes (Allport 2003). These instruments are widely used for their simplicity and instantaneous results, which is crucial in emergency situations (Allport 2003). Paper tape monitors are also useful tools in detecting leaks by their continuous and unattended operation that are capable of giving audible warnings at set thresholds (Allport 2003). However, these instruments were shown to under-sample aerosols, especially atmospheres with particle diameters greater than 5μm (Hext et al. 2003). In addition, erroneous results may also occur at very high or low humidity. Therefore, these instruments are not suitable for regulatory compliance.

Integrated sampling methods use either impingers containing a solution of a derivatizing agent, or a glass fiber filter coated with a derivatizing agent that are connected to a pump calibrated to a method-specific flow rate. Dissolutions or adsorptions are amenable mechanisms of isocyanate collection, which permit subsequent analysis by either gas chromatography (GC), or more commonly high-performance liquid chromatography (HPLC) (Streicher 1994). Different HPLC columns, detection systems, and elution solvents are interdependent upon the sampling objectives and techniques (Allport 2003; Rom and Markowitz 2007).

Both wet and dry (solventless) techniques have coevolved for sampling airborne isocyanates. One of the first methods developed was by Marcali in 1957 using a bubbler with a mixture of 0.4N HCl and 0.4N acetic acid as the absorbing medium (Marcali 1957). Sodium bromide was added in the sample preparation step to catalyze the diazotization of the amine. While the Marcali method was the method of choice for TDI and MDI, major limitations ignited extensive modifications to increase sensitivities and overcome interferences (Allport 2003).

Presently, impingers have replaced bubblers with specific dimensions for: jet diameter; jet-to-plate distance (or strike distance); and, flow rate, which determine the velocity of the aerosol in the impinger jet (Allport 2003; Grinshpun 1997). Sampling efficiency is dependent on these dimensions, as well as impaction and diffusion mechanisms of particles and gases, respectively (Allport 2003). Impingers have been the preferred method when new reagents have been introduced, such as the nitro reagent, MAMA, 1-2PP, 2-MP, tryptamine, DBA, and MAP (Keller, Dunlap, and Sandridge 1974; Sango 1980; Ellwood 1981; Hardy 1979; Warwick 1981; Wu et al. 1987; Spanne 1996; Rudzinski et al. 1996).

Diffusion is the governing property when sampling TDI, which shows greater than 95% sampling efficiency. Since TDI is mostly present as a vapor in the workplace, TDI molecules

diffuse to the surface of the solution upon entry into the jet and end up suspended in the reagent-containing solution. Additionally, Lesage et al. demonstrated that conditions in PUR spray applications, specifically residential construction, were ideal for impinger methods based on the size of MDI aerosols generated (Lesage et al. 2007). Briefly, most of the MDI particles in this study were greater than 2μm, which were readily trapped in solution. Dissolution of these airborne particulates in the solution facilitated derivatization of MDI for more accurate detection and quantitation.

Alternatively, impingers are unsuitable for conditions that generate aerosols less than 2μm, specifically 0.01 to 1μm, because neither inertial nor diffusion mechanisms affect particle motion in this size range (Allport 2003; Hinds 1999; Spanne 1999; Hext et al. 2003). Consequently, these particles penetrate the impinger causing substantial transmission losses (Streicher et al. 2000). Therefore, a cut-off efficiency of 2μm is recommended for impingers otherwise the collection efficiency drops off rapidly, which is intimately linked to the calculated particle diameter having 50% collection efficiency (d_{50}) of a particle size for a specific impinger (Hinds 1999).

Glass-fiber filter (GFF) methods are solventless approaches to airborne isocyanate sampling that offer a few advantages over impinger, or wet methods. For instance, the use of volatile solvents requires the impinger to be refilled due to evaporation (Streicher et al. 2000). Additionally, impingers are inherently hazardous as workers are potentially exposed to solvent vapor, and solvents are potentially flammable.

Reagent-coated GFFs are prescribed for isocyanate particles less than 2μm to maximize contact and mixing between analyte and the derivatizing reagent, typically 1-2PP (Streicher et al. 2000; Tucker 2007). Following completion of sampling process, the filter is removed from the

cassette sampler (e.g.,, 37mm) and desorbed with a derivatized isocyanate-miscible solvent. This reaction product is then isolated and identified by retention times on the chromatograph, and quantified by one of several HPLC methods using existing monomeric analytical standards.

Putatively, GFFs prevent passage of vapors and particles of widely varying sizes through the sampler relative to other collection methods (Dharmarajan 1979; Tucker 1982). However, efficient mixing between the isocyanate particles and the reagent is a key principle in the overall determination of airborne isocyanate exposure (Streicher 1994). Therefore, the derivatization efficiency is inversely related to the diameter of the aerosols. Accordingly, filters are recommended for sampling particles smaller than 2μm, especially because impingers inefficiently collect these particles. Smaller particles also require less reagent contact; therefore they are less susceptible to local depletion. Minimal dispersion of larger spray-paint droplets have been shown in micrographs to impede accessibility of the derivatizing reagent, and without stabilization, isocyanates are able to either cure or competitively react (Streicher 1994; Bell 1994).

Uneven distribution of a thin coating of derivatizing agent in the equatorial plane may contribute to the local depletion, and ultimate loss of isocyanate. In 2007, Tucker showed that methods for coating GFFs are not standardized and that a wide range of reagent quantities may be encountered (Tucker 2007). Two common methods include total immersion of the filter into a solvent containing the reagent, and application of a known volume of reagent. These two methods were applied in this study, and the filters were analyzed by flow-injection with a UV detector. For instance, twenty-one 5-mm circles were punched out of an SKC, Inc. GFF coated with 1-2PP and placed into separate vials. Results from the flow injection analysis reported a

30.8% relative standard deviation of measurement of 1-2PP, with a 3-fold difference between largest and smallest quantities.

The ACGIH, International Organization for Standardization, and the European Standardization Organization have adopted an inhalable convention, based on particle size and the region of particle penetration within the respiratory tract (Maynard 1997). Standards for aerosol inhalability were based on a penetration curve. Using mannequins in a wind tunnel, inhalability of aerosols was based on wind speeds between 1 and 4 m/s (Baldwin and Maynard 1998; Maynard 1997). Wind speeds in many indoor environments have been documented significantly below this range. Baldwin and Maynard surveyed wind speeds in indoor workplaces and found that wind speeds peak above 1 m/s, but over 85% of the measurements were below 0.3 m/s (Baldwin and Maynard 1998).

The aspiration efficiency of particles up to 20 μm have been collected with some degree of efficiency relative to the inhalability convention by 37 mm open- and closed-face cassettes (Bartley 1998). For larger particle sizes and relatively high wind speeds, 37 mm cassettes (and impingers) are expected to undersample relative to the inhalability convention.

Buchan et al. compared sampling efficiencies of open- and closed-face 37mm filter cassettes in a wind tunnel with various aerosol sizes, up to 24μm MMAD (Buchan, Soderholm, and Tillery 1986). In this study, mass concentration measurements in paired isokinetic samples were used as controls, which demonstrated a causal relationship between size and cassette sampling efficiency.

GFF sampling can be accomplished using open-, or closed-face techniques, each having limitations. For instance, results from GFF-cassette samplers may show positive or negative bias (Tucker 2007). Bias factors in closed-face aerosol sampling may be attributed to anisokinetic

conditions over-, or under-sample aerosols if the intake stream is faster or slower, respectively, relative to the air stream. Other factors governing may include, sampling angle, particle bounce, particle re-suspension, cassette inlet diameter, electrostatic effects, and cassette sealing problems.

Comparisons between GFFs and impingers have been conducted under laboratory conditions as well as in the field, which revealed high variability in collection efficiencies. Laboratory conditions are usually absent of competitive reactions unlike field conditions, and therefore kinetics, comparatively, are inconsequential (Streicher et al. 2000). As a result, GFFs have consistently reported higher results than impingers in laboratory evaluations and impingers tend to yield higher results in the field (Streicher, Kennedy, and Lorberau 1994). The derivatization rate is an important concept in isocyanate sampling, especially spray operations using fast curing MDI- or TDI-based products (Streicher et al. 2000).

A study by the International Isocyanate Institute (III) compared various sampling methods employed regularly for polymeric MDI using two concentrations, 0.1 and 1.0 mg/m^3, at aerosols of various aerodynamic diameters: 0.1-3µm (small); 5-20µm (large); >30µm (very large) (Hext et al. 2003). Small particles were over-sampled at 0.1mg/m3, while the impinger trapped large particles at a collection efficiency of 111% for 0.1mg/m3. Conversely, small aerosol particles (60-65%) passed through the impinger. Therefore, depending on the application of the isocyanate and the method of aerosol generation, the sampling results may not accurately represent true concentration of either MDI or TDI and over exposures may go unnoticed.

Lesage et al. compared results from a side-by-side comparison of impinger and filter samples collected during application of spray PUR foam inside five single-family homes under construction (Lesage et al. 2007). Applicator exposures were determined by personal sampling (n=13), which ranged from 0.07 to 2.05 mg/m^3. Approximately 70% of the 13 applicators

exceeded the OSHA PEL with some of the results as much as 10 times this exposure limit. Almost two-thirds of the particle mass concentration of the spray foam aerosol was greater than 3.5 microns in diameter, which resulted in up to 40% underestimations of airborne MDI concentrations by filter sampling methods compared to those determined by impinger methods.

Exposure assessment at ultratrace levels (1$\mu g/m^3$ or parts per trillion) is especially critical in order to protect sensitized workers (Gagne et al. 2005; Levine 2002). Ideally, a sampler should collect both gas and particles in a representative way, which is also capable of separating the analytes into separate size fractions (Dahlin et al. 2008; Streicher et al. 2000; Henneken, Vogel, and Karst 2007; Marand et al. 2005; Huynh 2009). Methods amenable to differentiating between vapor and aerosol exposures have been proposed due to the difference in penetration and deposition in the respiratory tract. Temporality and causality of health consequences may vary with these different types of exposure. The ISO-CHEK method was developed to simultaneously capture and separate vapor and aerosol phases of isocyanates using a dual filter system consisting of a front PTFE filter for the aerosol phase and a MAMA-impregnated GFF filter for the vapor phase (SKC). However, limitations exist that are attributed to losses and misclassifications of semi-volatile species, which a negative bias of 45% has been reported (Levine 2002). The decrement is worse for TDI than for MDI, especially with longer sampling times.

Other method developments capable of gas and particle separation have included: NIOSH's MAP Draft Method 5525, a sampler containing a reagent-coated PUR sponge, and an annular denuder-impactor system (Dahlin et al. 2008; Rudzinski et al. 2001; Bello, Streicher, and Woskie 2002). The NIOSH draft method 5525 was introduced to meet the terms of TRIG analysis, which utilizes the reagent 1-(9-anthracenylmethyl) piperazine (MAP), which is a strong

chromophore and fluorophore with high sensitivity and selectivity (Levine 2002). Additionally, the denuder-impactor system is too large for personal sampling, and therefore suffers from a lack of practicality and ease of use (Streicher et al. 2000).

The commonly used sampling methods that are the focus of this study are the OSHA Sampling Method 47 for MDI and 42 for TDI, and the Wisconsin Occupational Health Laboratory (WOHL) MDI/TDI Sampling Method LC 48 (Administration ; Laboratory). The OSHA Sampling Methods are based on laboratory desorption (LD) of the filter; both the cassette and filter are mailed to an analytical laboratory where extraction occurs in a solution of 90% acetonitrile and 10% dimethyl sulfoxide (90/10 ACN/DMSO) (Administration ; Administration). On the contrary, the WOHL Sampling Method dictates that either monomer samples are field desorbed (FD) (*immediately* after sampling) in a collection vial containing two milliliters of 90/10 ACN/DMSO and then sent for analysis (Laboratory). Both LD and FD extraction solutions are injected onto a packed, high-performance liquid chromatography (HPLC) column interfaced with an ultraviolet detector for analysis and quantitation of MDI and TDI.

Both the OSHA and WOHL sampling methods require drawing air through an open-face cassette containing a glass fiber filter coated with 1.0mg and 0.1mg of 1-(2-methoxyphenyl)-piperazine (1-2PP) for MDI and TDI, respectively. 1-2PP (a secondary amine) is a derivatizing agent coated on the sample filters that facilitates the analytical detection of the isocyanate functional group by improving sensitivity and selectivity of MDI and TDI. 1,2-PP was introduced in 1979, which replaced earlier pioneered methods based on either ethanol or nitroreagent serving as the derivatizing agent (Hardy 1979). The nitroreagent was light sensitive, and readily degraded when exposed to sunlight and fluorescent light. Consequently, degradation products of the nitroreagent interfered with chromatographic analysis (Allport 2003).

The physicochemical properties of 1-2PP afforded greater stability (e.g.,, 283°C bp), a wider range of miscibility in polar and non-polar solvents (including water), and less susceptibility to light degradation than the nitroreagent (Hardy 1979). With negligible steric hindrance, isocyanate groups react rapidly. Ideally, 1.2-PP prevents competitive loss of the NCO group to polyols and/or water, and the resulting urea derivatives are detectable at low concentrations (Streicher et al. 1996).

Originally, impinger techniques were validated using a $2x10^{-4}$ M solution of 1-2PP in toluene (Allport 2003). Thin-layer chromatography and HPLC methods were developed for detecting 1-2PP derivatives by either fluorescence or UV.

OSHA modified the method, substituting a 37-mm GFF impregnated with 1-2PP for the impinger device. Extensive validation was performed in order to ratify Methods 42 and 47 for TDI and MDI, respectively, with the caveat that liquid spiking was used instead of aerosol generation for MDI Method 47. Spiking a solution of analyte does not accurately reflect field conditions of the derivatizing reaction because the isocyanate is spread out evenly over the filter area. Depending on the environmental conditions during sampling and face velocity of the cassette, stacking of particles may occur with limited contact between isocyanate and reagent. Additionally, liquid spiking gives mobility to the derivatizing reagent, and at least briefly provides a solution environment for derivatization to occur.

Anecdotal evidence gathered in evaluations of 1-2PP for the sampling and analysis of airborne isocyanates revealed that the reagent readily sublimes from the GFF during long sample times, especially in hot, humid weather (Allport 2003). One group determined that as much as 67% of the 1.0 mg of reagent coated on the filter evaporated off after a four-hour sample time

that was initiated to comply with the 1989 PEL, which increased the sampling air volume from 15L to 240L.

Another study demonstrated capillary transfer of 1-2PP as high as 85% in 24 hours from the GFF to a mixed cellulose ester (MCE) backup pad. Corrective actions of suspending the filter in the middle of the cassette with no back-up pad, or replace MCE with stainless steel were recommended.

In an MDI stability study by Karoly, samples were collected on 13-mm glass fiber filters (GFF) containing the derivatizing agent MOPP and 2% diethyl phthalate using a company-specific sampling and analytical method (ICI Polyurethanes sampling and analytical Method I 1024G, revision 1.7) (Karoly 1998). Side-by-side samples were collected at four different wood mills from one to three hours and sample preparation included one field desorbed (FD) and one laboratory desorbed (LD) filter within each sample set. Briefly, the FD filters were removed from the cassette, rinsed, and immersed in a vial containing derivatizing agent and toluene. LD samples were sealed in the cassette to be desorbed at the laboratory. Both sets of samples were sent to an analytical laboratory.

Samples that were FD yielded statistically significant higher amounts of the isocyanate as compared to LD samples. These results indicate the impact of dusty environments on filter paper methods and the attendant underestimation of MDI airborne concentrations when LDs were performed. FD samples, most likely, promoted efficient mixing between MDI-coated dust particulates and MOPP and prevented loss of hindered isocyanate to other competitive reactions (e.g.,, excess polyol).

While Karoly's study reveals methodological limitations in MDI sampling, these results are based on proprietary methods in a dusty environment using the derivatizing agent MOPP.

In a preliminary study, we compared two sets of MDI field-data collected side-by-side from WOHL Sampling Method 48 (field desorbed) and OSHA Method 47 (laboratory desorbed) to evaluate results of the same contaminated atmosphere for reproducibility (see Preliminary Studies). Both methods, which use 1,2-PP as a derivatizing agent, generated significantly different results. We hypothesize that a statistically significant difference will also exist in TDI field-data collected by OSHA Sampling Method 42 and the WOHL Sampling Method LC 48. However, it is crucial to acquire independent, field- and controlled-data due to the relative difference in reactivity between the congeners and 1,2-PP to evaluate OSHA and WOHL Methods for accuracy of airborne MDI and TDI.

Kuck, et al. investigated partial rate factors between monomeric diisocyanates and six different derivatizing reagents in test solutions. Reaction rates were shown to vary by orders of magnitude between diisocyantes. Inherent properties such as: selectivity, stability, sensitivity, and geometry of both the agent and diisocyanate likely account for such reactivity differences (Kuck 1999). Rapid diisocyanate derivative stabilization is a key step in the prevention of isocyanate loss due to side reactions with other compounds or artifacts. Consequently, reaction rates have the potential to significantly impact the accuracy of analytical determinations of airbore diisocyanate monitoring (Kuck 1999).

Tremblay, et al. evaluated competitive rates of four different derivatizing agents with aliphatic and aromatic diisocyanates (Tremblay et al. 2003). Relative reactivity was demonstrated to be a function of the chemical structure of the diisocyanate, even between TDI isomers (e.g., 2,4- and 2,6-TDI). Using specific software, physicochemical properties (including electron density and electrophilicity) of both TDI functional groups were found to be analogous, attributing relative reactivity to the three-dimensional difference of each TDI isomer. Since the

2, 6-TDI isomer is an asymmetrical molecule; the methyl group substituted at the one position on the benzene ring causes equal hindrance on the isocyanate groups. Consequently, 2,4-TDI is much less hindered, and yields a greater derivatization percentage. Structural differences were additionally noted in the Tremblay et al. investigation between MDI and TDI with secondary amines. Such differences in reactivity were examined by the formation of the urea derivative and the corresponding UV response. Varying response factors and retention times of MDI and TDI were easily observed when individual chromatograms were overlaid on each other.

Relevant competitive rate studies have emphasized a disparity in reactivity between MDI and TDI with a gamut of secondary amine derivatizing agents (Streicher, et al., 1996; Kuck, et al., 1999; Wu, et al., 1991). However, anecdotal variations still exist between MDI and TDI in relation to 1,2-PP, as this secondary amine was not evaluated. Therefore, it is very important that additional investigations are conducted which accurately assess the potential for disparity between MDI and TDI reactivity with 1,2-PP, specifically analyzed by both field and laboratory desorbed sampling methods.

While variants of sampling methods for MDI and TDI exist, companies have greater incentive to use the OSHA methods in order to comply with current regulation. However, OSHA and WOHL methods differ in their post-sampling procedures, which may prove to be a methodological limitation or weakness. Ultimately, a quantitative comparison will be based on urea derivative formations, proportional to MDI and TDI consumption in a controlled setting. These results will not only designate a more accurate method for monomeric MDI and TDI exposure, but will also emphasize the difference in relative reactivity between MDI and TDI with the derivatizing agent 1,2-PP. Such results will directly impact which sampling method an occupational health practitioner may use to accurately monitor worker exposures. As a result,

appropriate and effective control measures may be implemented that mitigate the exposure to a protective metric.

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CHAPTER 2—DEVELOPMENT AND CHARACTERIZATION OF A NOVEL ISOCYANATE AEROSOL GENERATING SYSTEM FOR EVALUATING EFFICACY OF DESORPTION TECHNIQUES IN GLASS FIBER FILTER SAMPLER

SUMMARY

The goal of this project was to develop and evaluate an isocyanate aerosol generating system that could be used to critically evaluate the accuracy of glass fiber filter samplers, which are commonly used to monitor a worker's exposure in the field. This aerosol generating system was designed to emit a wide range of isocyanate aerosols to effectively model the typical range of exposures encountered in polyurethane spray applications. The initial validation of the delivery system, a KD Scientific 200 Two-Syringe Infusion Pump interfaced with an EZ-STARTER airbrush with an atomizing nozzle from PAASCHE, was completed using water to ensure design values and expectations were achieved. The reported accuracy and reproducibility of the KD Scientific 200 Two-Syringe Infusion Pump were ±<1% and ±<0.1%, respectively. Dispensed flow rates of 0.193 and 0.380 ml $min.^{-1}$ were validated with an experimental value of less than 1% error emitted from the isocyanate aerosol generating system. A subsequent pilot study, consisting of five samples, was conducted using a working solution corresponding to 1.3 mg of methylene bisphenyl isocyanate per ml of toluene, which was dispensed at a flow rate of 0.193 ml $min.^{-1}$. This pilot study was a proof of concept and not an evaluation of the glass fiber filter method. With a standard deviation of 18 nanograms and a coefficient of variation of 8%, the isocyanate aerosol generating system exceeded expectations of consistent delivery of aerosolized methylene bisphenyl isocyanate. Toluene did not interfere with derivatization based on a series of dilutions using a fixed amount of methylene bisphenyl isocyanate with an increasing volume of toluene. Additionally, particle size distribution of the isocyanate aerosol generating system was characterized using a Grimm Dust Monitor Model 1.108. Data were

approximately log-normally distributed and count median-, and mass median diameters were calculated for each MDI working solution. The number and mass of particles cm^{-3} was normalized to the size range collected in each channel. An interval-normalized number and mass frequency plot were constructed to summarize the distribution. Approximately 95% of the aerosol mass concentration was associated with particles greater than 2 μm while 95% of the aerosol number concentration was associated with particles less 2 μm. The majority of the number concentration (75%) was contained between 0.35 and 0.725 μm. Particles greater than 3.5 μm contained 75% of the mass during the process. Some interesting effects of particle size and methylene bisphenyl isocyanate concentration were observed in this study. Notably, the mass median diameter decreased with increasing mass concentrations of isocyanate. Since 2 μm is the recommended upper limit for using glass fiber filters, ideally, the MMD would have been closer to 1μm to evaluate accuracy of sampling airborne isocyanates. However, the isocyanate aerosol generating system has provided a basis to model future experiments that spray load filters with known amounts of isocyanate. Overall, this project demonstrated that an isocyanate aerosol generating system could be developed for the purpose of spray loading glass fiber filters with known amounts of analyte to evaluate accuracy.

INTRODUCTION

Diisocyanates (monomers) are essential precursors used extensively to manufacture a wide range of polyurethane end products (Lesage et al. 2002; Boutin et al. 2005; Karol 1986). These monomers are bi-functional, characterized by the presence of two isocyanate (NCO) functional groups attached in varying positions to an aromatic or saturated (aliphatic or alicyclic) parent compound (Streicher et al. 2000; Deschamps et al. 1998; Nakashima 2002; Weyel and Schaffer 1985; Bello, Herrick, et al. 2007; Woolrich 1982; Bello et al. 2004). Accordingly,

diisocyanates are inherently reactive chemicals capable of polymerization. This reactivity contributes to the technical value of diisocyanates, as well as their toxicity (Ott et al. 2007; Raulf-Heimsoth and Baur 1998). Consequently, diisocyanates are one of the most common causes of occupational asthma (Wisnewski and Jones 2010).

Polyurethanes derived from diisocyanates have a multitude of industrial and consumer applications. For example, diisocyanates can be used to produce a broad spectrum of polyurethanes used in coatings, adhesives, sealants, and elastomers (CASE). These products are durable against temperature and weather extremes, and resistant to ultraviolet light and chemicals (De Vries et al. 2012; Boutin et al. 2005). Additionally, CASE products are easily molded into any shape, lighter than metal, and offer superior stress recovery. Collectively, these features are quite useful to the automotive, aerospace, and construction industries. Other diisocyanate-derived polyurethanes, such as glues and binders are used to make engineered wood products (Booth et al. 2009).

In the year 2000, global production of polyurethanes exceeded 9 billion kg (Krone 2004). More than 4 billion kilograms (kg) of diisocyanates are produced worldwide annually in order to maintain such a large-scale production. Commercially, two of the most important isocyanates are: 2,4-, and 2,6- toluene diisocyanate (TDI), and 4,4' methylene bisphenyl isocyanate (MDI) (Bello et al. 2004). These aromatic isocyanates account for over 90% of the diisocyanate market (Raulf-Heimsoth and Baur 1998; Allport 2003).

MDI and TDI are used to manufacture both flexible and rigid polyurethane foams; however, the foamed polyurethane industry is rapidly changing on a global scale. For instance, in 1999, the Alliance for Flexible Polyurethane Foam reported an annual production of over 2.1 billion pounds in the US annually (Krone et al. 2003). Beginning in 2008, production of these

foams protracted as a result of the global financial crisis (Global Industry Analysts 2013). Currently, production growth of foamed polyurethanes is projected to reach 9.6 million tons by the year 2015. This resurgent demand is driven by the elastic demand of polyurethanes in the construction, furniture and bedding, and automotive markets in Europe, Asia-Pacific, and United States (Global Industry Analysts 2013).

Domestic production capacity was growing at a rate of 2.1% per year through 2004 and this trend is likely to continue at a high level as new markets and applications continue to develop (Allport 2003; Program 2009). With an aging population, the need for low-cost materials, such as diisoycanates, in the development of new technology in mattresses is highly anticipated, especially in aging health conscious populations (e.g., Asia-Pacific regions) (Global Industry Analysts 2013). Flexible polyurethane foams are the key component of cushioning material found in automobile seating, and carpet pads due to their elastic properties; however, the furniture and bedding industry represent the largest end-use market of these foams (Krone et al. 2003; Cummings and Booth 2002; Global Industry Analysts 2013).

The movement towards energy efficient appliances and buildings will foster growth in the rigid foamed polyurethane market (Global Industry Analysts 2013). Rigid polyurethane foams deliver a unique combination of thermal stability (e.g., low thermal conductivity) and mechanical properties (e.g., high compression strength, high strength-to-weight ratio, and low moisture permeability) that drives their versatility as insulation for refrigerators, freezers, piping, tanks, and shipbuilding (Kim 2008). Recently, builders have exploited these properties to reduce heating and cooling costs by maintaining uniform temperature through high performance insulation and air barrier sealants (Council 2005).

The inherent reactivity of diisocyanates is explained using the molecular orbital theory that predicts the greatest charge density on the oxygen atom, which imparts the highest net positive charge on the carbon (Arnold 1957). The major resonance structure (Figure 2-1) of diisocyanates accounts for the high reactivity and wide spectrum of reactions of isocyanates. For example, diisoyanates promote polyaddition-polymerization through heteroatom cross-linking of a co-monomer possessing a reactive hydrogen atom (e.g., -OH, -COOH, -NH2, -SO2NH2) (Booth et al. 2009; Malten and Zielhuis 1964).

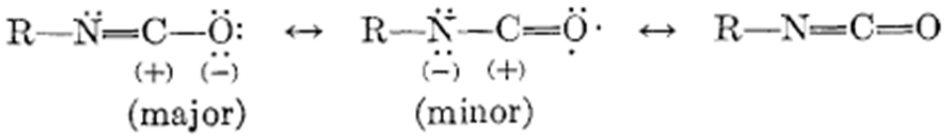

FIGURE 2-1. Resonance structures of isocyanates (Arnold 1957).

Any active hydrogen compound can react with the isocyanate groups to produce addition compounds by a nucleophilic attack upon the electrophilic carbon (Ashida 2007; Arnold 1957). Reactivity of the X—H and NCO moiety increases as nucleophilicity and electrophilicity increases, respectively (Arnold 1957). For example, when stronger electron-withdrawing groups are attached to isocyanate entity, a greater positive charge will be placed on the carbon. Typically, the co-monomer involved in polyurethane production is a polyfunctional alcohol, known as a polyol (e.g., polyether polyol).

Polyols are compounds with multiple hydroxy functionality that serve as nucleophilic agents that react instantly with diisocyanates in a one-step polymerization process (Ulrich 1996). Polyols promote a concerted, nucleophilic addition across the N=C bond of the isocyanate (Raspoet et al. 1998). For example, ethylene glycol is a molecule with two hydroxy groups, also known as a diol. The hydroxy functionality of at least two allows for addition across the N=C double bonds to form repeat units of NHCOO groups characteristic of polyurethane products

(Ashida 2007). The chemical backbone of polyols varies significantly (e.g.,, polyester, polyether, polyester-polyether hybrids, or hydroxyl-containing vegetable oil) according to dimension specifications needed of the polymer (Ulrich 1996; Ashida 2007).

In general, exposure potential for MDI and TDI is interdependent on their vapor pressure and use. Accordingly, potential for vapor exposure to MDI is low, as it does not readily volatilize at ambient temperatures. However, if mechanically atomized or heated in the work environment, vapors and aerosols with wide range of particle sizes will pose as an inhalation hazard (Booth et al. 2009). On the other hand, vapor pressure of TDI is orders of magnitude higher; therefore exposures to both TDI vapor and aerosols are possible depending on expected application of the product (Cummings and Booth 2002; Laboratory).

Workers are potentially exposed to unreacted diisocyanate monomers, prepolymers, oligomers, and/or polyisocyanate species depending on the formula and application of the isocyanate product (Streicher et al. 2000). While toxicity differences between these isocyanate-related species most likely differs due to factors beyond inherent reactivity of the NCO functional group such as physicochemical properties (e.g., electrophilic, lipophilic, three-dimensional structure) of the parent compound attached to the NCO group, deposition site in the lungs, or concomitant exposures (e.g., mixtures of monomers and polyisocyanates, solvents), there is limited body of literature consisting of animal and human data on polyisocyanate exposures (Bello et al. 2004). Accordingly, there is no occupational exposure limit for polyisocyanates mandated in the US. The United Kingdom Health and Safey Executive regulate isocyanate exposure using total NCO mass as the exposure metric.

According to a National Occupational Exposure Survey, approximately 280,000 US workers are potentially exposed to MDI and TDI; however, this figure does not accurately reflect

the ubiquity of diisocyanate-based consumer products, such as do-it-yourself polyurethane spray foam and paints (Health. 2006; Wisnewski et al. 2012). The National Institutes of Health Household Products database lists 16 MDI-containing home-maintenance sealants and adhesives that contain between 4% and 70% MDI (Services). Nonoccupational and bystander exposures to isocyanates are growing as a result of residential or public area application(Krone 2004; Wisnewski et al. 2012). Jan et al. examined previously unexposed school children for acute asthma-like signs and symptoms following an accidental spill of MDI in the vicinity of a school. These authors detected an appearance of an acute asthma-like syndrome from pulmonary function tests and HPLC analysis of urine for the presence of residual MDI.

Exposure may also occur from combustion or thermal degradation of finished polyurethane products, as well as contact with slow curing isocyanates (Rom and Markowitz 2007; Boutin et al. 2005; De Vries et al. 2012). Free isocyanates are liberated under conditions of heat and abrasion, such as welding, grinding, and cutting/sanding. Bello et al. recently showed that auto body workers were in frequent contact with unbound NCO functional groups contained in polyurethane coated car surfaces (Bello, Sparer, et al. 2007). While the coated surface may appear dry, these unbound isocyanates may persist as drying times range from 5 minutes to 16 hours depending on the product and task. De Vreis et al. quantitatively examined the transferability of isocyanates from such surfaces using a test panel sprayed with isocyanate coatings and paired skin wipe samples from participants that came into contact with the recently dried surfaces (De Vries et al. 2012). Factors such as cure time and tasks other than spraying, such as compounding, were also evaluated. While approximately 84% of the test panels exhibited unbound NCO groups that were quantifiable, the risk of substantial skin exposure with these dried surfaces was concluded low. Skin wipe samples were obtained from 18 workers in

five auto body shops, and of the 104 non-compounded skin samples collected post-contact with dried surface, seven samples reported detectable levels of isocyanate using the NIOSH method 5525. Additionally, only 1 of 12 skin samples obtained after compounding reported detecteable levels.

Occupational exposures to MDI and TDI elicit serious worker health effects through interplay of inflammatory-, and immune-mediated mechanisms (Rom 1998). Occupational exposure to MDI presents serious worker health concerns as it may lead to either short- or long-term health effects such as asthma, airway irritation, hypersensitivity pneumonitis, and irritation of skin and mucous membranes (Chester et al. 2005; Lushniak et al. 1998). For some sensitized individuals, acute exposure may prove fatal (Lofgren et al. 2003). Inhalation is the most significant route of exposure due to the chemical and physical properties of MDI; specifically, heating and spraying the two-component STBL product promotes volatilization and mechanical aerosolization of the MDI.

There is growing evidence in the current literature that strongly suggests that dermal exposure plays a pivotal role in sensitization and asthma development (Bello et al. 2004). Skin exposure to isocyanates may cause contact dermatitis (both irritant and allergic) with symptoms such as rash, itching, hives, and swelling of extremities (Bello et al. 2004; Streicher 1994). Workers may experience mild symptoms of occupational rhinitis characterized by episodic work-related sneezing, nasal discharge, and nasal obstruction; as well as eye irritation, coughing, and short of breath (Allport 2003; Merchant et al. 1986).

Occupational asthma, an inflammatory disorder of the airways, is the most prevalent respiratory illness attributable to MDI and TDI exposure (Rom 1998). Isocyanate-induced asthma follows a complex pathogenic mechanism as these low-molecular weight compounds

may induce allergic or irritant asthmatic responses, or both that are with-, and without a period of latency (Figure 2-2) (Rom 1998; Pronk et al. 2007).

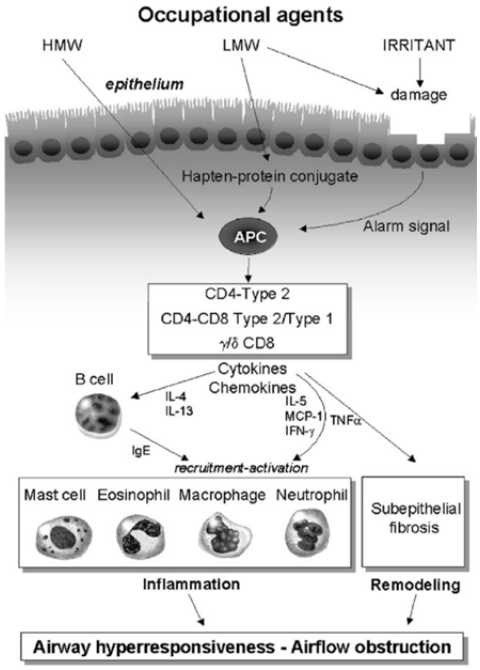

FIGURE 2-2. Overview of the mechanisms involved in the pathogenesis of occupational asthma mediated by both high molecular weight and low molecular weight agents (Mapp et al. 2005). As shown in this figure, asthma is a heterogeneous disease with respect to immunopathology and environmental causes (Mapp et al. 2005; Holgate 2008; Borish and Culp 2008).

Other adverse effects to the respiratory system include hypersensitivity pneumonitis (HP), and reactive airways dysfunction syndrome (RADS), and possibly accelerated pulmonary function loss (Allport 2003; Pronk et al. 2007; Wegman et al. 1977; Leroyer et al. 1998; Schreiber et al. 2008; Baur 1995).

To control monomeric MDI airborne exposures, occupational exposure limits are calculated by regulators, academic bodies, or industry from pooled resources including experimental animal studies, human data from clinical cases and epidemiological studies, chemical structure-biological activity models, and chemical-specific toxicology data (Allport

2003; DiNardi and American Industrial Hygiene Association. 2003). For example, the ACGIH recommends a threshold limit value (TLV), eight-hour time-weighted average (TWA) of 0.051mg/m^3 (5 ppb) [22]. This occupational exposure limit, while not enforceable, refers to a level that nearly all employees may be exposed to over a working lifetime without adverse health effects. Additionally, the NIOSH published a recommended exposure limit (REL) of 0.05mg/m^3 (5 ppb) and a ceiling limit of 20 ppb for up to a 10-hour workday during a 40-hour workweek [23].

The OSHA mandates a permissible exposure limit (PEL) for airborne, monomeric MDI and TDI of 200 and 140 micrograms per cubic meter (μg/m^3), which is equivalent to 20 parts per billion (ppb) (Administration). However, these PELs are based on an antiquated TLV list, which prescribes exposure criteria for MDI and TDI monomers only using a ceiling limit of 20 ppb, respectively (Bello et al. 2004; Administration). OSHA defines a ceiling value as an exposure limit that shall not be exceeded at any time during the working day for a particular substance. OSHA does not mandate a TWA standard for MDI or TDI; however, the protocol for monitoring MDI and TDI is to assess the ceiling over a 15-minute period (Bello et al. 2004). Consequently, instantaneous concentrations may exceed the ceiling limit, while the average may very well be within the legal requirements of 20 ppb for airborne aromatic diisocyanates. This underestimation is an important caveat in regulatory compliance and sampling strategy for both MDI and TDI since peak exposures may be more relevant than lower long-term, or cumulative exposures in terms of risk for developing symptoms of asthma (Allport 2003; Mapp et al. 1988; Mapp et al. 2005; Chan-Yeung and Malo 1995).

Accurate measurements of MDI and TDI in workplace atmospheres are crucial, especially for previously sensitized workers. Quantitative determinations rely on capturing a

representative sample; however, isocyanates characteristically have a widely varying disposition in the workplace, especially in spray applications (Pronk et al. 2006; Redlich and Karol 2002; Rom and Markowitz 2007; Streicher et al. 2000; Allport 2003; Bello et al. 2004). Therefore, isocyanate sampling and analysis is challenging (Streicher 1994; Streicher et al. 2000). Technical difficulty in quantitative determinations of MDI and TDI are due to episodic exposure profiles, high reactivity and adsorptivity to particulate matter, particle size and concomitant exposure to other isocyanate species (depending on the product formula), solvents, and other interfering additives (Allport 2003; Streicher et al. 2000; Redlich and Karol 2002; Lesage et al. 2007; Streicher 1994).

Exposure scenarios may vary considerably between TDI and MDI due to a saturated vapor pressure difference of several orders of magnitude. Kupczewska-Dobecka et al. reported the vapor pressure of MDI to be approximately 300 times lower than TDI (Kupczewska-Dobecka, Czerczak, and Brzeznicki 2012). The physicochemical properties of isocyanates permit them to exist as a vapor or aerosol with a wide range of particle sizes (Allport 2003; Streicher 1994; Streicher et al. 2000). While MDI does not readily volatilize at room temperature unlike TDI, certain processes may heat the isocyanate creating a vapor inhalation hazard. Additionally, the reaction between MDI and a polyol is exothermic, consequently providing the heat necessary for volatilization (Streicher et al. 2000). Therefore, end-users that spray MDI-based systems may encounter aerosol exposures from mechanical atomization of the material, as well as vapor exposures during exothermic reactions.

Isocyanate cure rate, or half-life, is an another important factor that must be considered when sampling diisocyanates (Streicher 1994). Curing is the consumption of unbound and unreacted isocyanates by free hydroxyl groups, which forms polyurethane polymers (Agency

2011). Completely cured products are considered non-toxic, inert materials that do not off-gas due to the fully reacted NCO groups. Cure rates may range from seconds to hours depending on the form of isocyanate used in the reaction, as well as the physicochemical properties of the co-reactant (e.g., two-component systems) (Streicher 1994). Once sprayed, two-component systems are considered to have a fast cure rate due to the presence of a catalyst (i.e., less than a few minutes). Alternatively, the use of diisocyanates as an adhesive (e.g., bonding two pieces of wood together without a catalyst) may have a cure rate of several hours.

To address this spectrum of exposure scenarios, a compilation of passive and active sampling methods has been developed to determine worker exposure to isocyanates. Passive sampling, specifically dosimeter badges, has been a practical tool based on worker acceptance, convenience, ease of use, and low costs (Levine 2002; Henneken, Vogel, and Karst 2007). Batlle et al. reported the use of solid-phase microextraction (SPME) with on-fiber derivatization as a passive sampler with a wide linear range and applicable limits of detection for gaseous TDI; however, the derivatizing agent used readily evaporated (Batlle, Colmsjo, and Nilsson 2001). Additionally, sampling rates are temperature-, and humidity-dependent since they rely on diffusion coefficients (Henneken, Vogel, and Karst 2007). Therefore, diffusive sampling devices are most effective as a screening tool and not quantitative determinations.

Direct-reading instruments, such as vapor detection badges and colorimetric tubes, provide continuous, unattended operation that are capable of audible warnings at set thresholds (Allport 2003). This technology has proved valuable for detecting leaks, and more importantly during emergency situations when instantaneous results are critical to ensure employee health and safety. While easy to use, direct-reading instruments have been shown to underestimate aerosols, especially with particle diameters greater than 5μm (Hext et al. 2003). Ideally, a

sampler should collect both phases of isocyanates (i.e., vapor and aerosol) while attaining a particle size distribution (Dahlin et al. 2008; Streicher et al. 2000; Henneken, Vogel, and Karst 2007; Marand et al. 2005; Huynh 2009).

In 1957, Marcali developed one of the first active sampling methods that used a bubbler with a solution of hydrochloric and acetic acids(Marcali 1957). Isocyanate levels were determined by colorimeteric analysis in the laboratory by adding sodium bromide to catalyze diazotization of the amine. In 1985, Rando et al. modified timing of diazotization and temperature in the Marcali method due to positive interferences with common chemicals present in the use of isocyanates, as well as lack of specificity between isocyanates (Rando and Hammad 1985). These proposed changes reduced an isomeric effect observed in monitoring TDI. For example, 2,4-TDI has a higher absorption coefficient than 2,6-TDI at the wavelength used in the Marcali method (Levine 2002). Studies in flexible slabstock foam facilities have shown exposures to 2,4-TDI are higher in the initial stages of the process while 2.6-TDI exposures are predominantly at the end stage (Levine 2002; Rando, Abdel-Kader, and Hammad 1984; Boeniger 1991; Cummings and Booth 2002).

Sampling methods have been evolving as knowledge has expanded on the complex matrix of airborne isocyanate exposures. In order to meet the need for lower limits of detection and quantitation, as well as less compound-to-compound variability, other consideration has been given to sample collection errors. Aspiration, internal wall and transmission losses exist in each sampling method, which impact the capture efficiency of particulate isocyanates (Streicher et al. 2000; Tucker 2007; Allport 2003). Aspiration errors may introduce a positive or negative bias in the results and not accurately reflect human inhalation efficiency. This error occurs when the probe intake stream does not match the velocity of the air stream. Internal wall losses account

for deposition of particles along the internal components of the sampler (e.g., inlet and nozzle of impingers, and filter holder) that are not available for subsequent desorption and analysis. Transmission losses from sample media can occur from breakthrough or complete penetration of particles.

Currently, active sampling is typically achieved by use of an impinger filled with an absorbing solution or a reagent-coated glass fiber filter (GFF) sampler. These two sampling systems contain a derivatizing agent to enrich analytical identification and quantitation of isocyanates based on the formation of a stable urea derivative with strong molar absorptivities [5, 22].

Derivatizing agents are typically primary or secondary aliphatic amines. [5] Common derivatizing agents include tryptamine, 1-(2-methoxyphenyl) piperazine (MOPP), and 1-(9-anthracenylmethyl) piperazine (MAP) as listed in the NIOSH Manual of Analytical Methods for isocyanates; 9-(methylaminomethyl) anthracene (MAMA), which is used in the SKC Inc. Iso-Chek method; and, 1-(2-pyridyl) piperzine (1-2PP) as used in OSHA Method 47 for MDI (Products ; SKC ; Administration ; Administration).

Original impinger techniques were validated using a $2x10^{-4}$ M solution of 1-2PP in toluene (Allport 2003). Thin-layer chromatography and HPLC methods were developed for detecting 1-2PP derivatives by either fluorescence or UV. Hardy and Walker introduced 1,2-PP in 1979, which replaced earlier derivatizing agents, ethanol and nitroreagent (Hardy 1979). Analytical detection of the isocyanate functional group was enhanced with the development of 1,2-PP by improving sensitivity and selectivity of MDI and TDI. For example, the nitroreagent was light sensitive, and readily degraded when exposed to sunlight and fluorescent light.

Consequently, degradation products of the nitroreagent interfered with chromatographic analysis (Allport 2003).

1,2-PP prevents competitive loss of the NCO group to polyols and/or water, and the resulting urea derivatives are detectable at low concentrations (Streicher et al. 1996). The physicochemical properties of 1-2PP afforded greater stability (e.g., 283°C bp), a wider range of miscibility in polar and non-polar solvents (including water), and less susceptibility to light degradation than the nitroreagent (Hardy 1979). With negligible steric hindrance, isocyanate groups react rapidly.

In 1981, Warwick et al. introduced the derivatizing agent MOPP for the determination of isocyanates, which allowed photometric and electrochemical detection with good sensitivity and limits of detection down to 0.2 $\mu g/m^3$ (Warwick 1981). The NIOSH Method 5505 uses this derivatizing agent in an impinger, and analysis is accomplished using an HPLC. Quantitation is accomplished by comparing the starting amount of MOPP to the remaining amount after the sample has been collected.

While a variety of derivatizing agents, and sampling and analytical methods exist for measuring airborne concentrations of isocyanates in the workplace, certain methodologies are preferentially selected depending on the process. The NIOSH recommends an impinger method, specifically NIOSH Method 5525, during work operations that spray fast-curing isocyanates, especially when sampling times are not adjusted to the product half-life (Streicher 1994; Streicher et al. 2000; Lesage et al. 2007). Fast-curing isocyanates may be lost to competitive reactions that can occur between collection and post-sampling procedures instead of reacting with the derivatizing agent. The impinger uses a solvent medium to trap, dissolve, and stabilize the isocyanate aerosols to help prevent an underestimation of isocyanates (Streicher et al. 2000).

The use of excess reagent in the solution enhanced derivatization kinetics and quantitative recovery. Flow rate and specific dimensions of the impinger (e.g., jet diameter and strike distance) govern sampling efficiency, as well as impaction and diffusion mechanisms of particles and gases (Allport 2003).

Impinger methods are also recommended for processes that generate particles greater than 2μm (Streicher 1994; Streicher et al. 2000). Impingers prevent the passage and allow dissolution of isocyanate particles greater than 2μm. A study by the International Isocyanate Institute (III) compared various sampling methods employed regularly for polymeric MDI. Two concentrations, 0.1 and 1.0 mg/m^3, of aerosols were produced with various aerodynamic diameters: 0.1-3mm (small); 5-20mm (large); >30mm (very large) (Hext et al. 2003). Over seven samplers were evaluated in this study at each concentration and particle diameter range. Samplers of particular interest were GFFs of various sizes and mini-impinger. Using an isokinetic reference sampler, collection efficiencies of each test sampler were determined. Compared to the reference sampler, GFFs over-sampled (i.e., collected higher amounts of MDI than the reference sampler) the small particles at a concentration of 0.1mg/m3. On the other hand, the impinger over-sampled (e.g., collection efficiency greater than 100%) the large particles at both concentrations of aerosols. Conversely, small aerosol particles (60-65%) passed through the impinger. Therefore, depending on the application of the isocyanate and the method of aerosol generation, the sampling results may not accurately represent true concentration of either MDI or TDI and over exposures may go unnoticed.

Although impinger methods are preferred, their use is often inconvenient and may prove unsuitable for certain conditions. For instance, volatile solvents used in the impinger evaporate and must be refilled. Additionally, impingers are inherently hazardous as workers are potentially

exposed to solvents such as toluene and dimethyl sulfoxide. Furthermore, some impinger solvents are flammable.

OSHA modified the original impinger method by substituting a 37-mm GFF impregnated with 1-2PP for the impinger device. Since OSHA has the authority to enforce occupational exposure limits, isocyanate personal samples are usually collected using this method. OSHA recommends drawing a known volume of air through an open-face cassette at 1 liter per minute (L/min) for a total volume of 15 liters (L). The OSHA Sampling Method 47 for MDI uses 1.0 milligrams (mg) to coat the GFF while the OSHA Sampling Method 42 for TDI uses only 0.1mg of the secondary amine (Administration ; Administration).

Anecdotal evidence gathered in GFF evaluations of 1-2PP while sampling airborne isocyanates revealed several limitations. One group determined that the reagent readily sublimes from the GFF during long sample times, especially in hot, humid weather (Allport 2003). As much as 67% of the 1.0 mg of reagent coated on the filter evaporated off after a four-hour sample time that was initiated to comply with the 1989 PEL, which increased the sampling air volume from 15 L to 240 L. Another study demonstrated capillary transfer of 1-2PP as high as 85% in 24 hours from the GFF to a mixed cellulose ester (MCE) backup pad. Recommended corrective actions included suspending the filter in the middle of the cassette with no back-up pad, or replacing the MCE with a stainless steel backup pad.

The OSHA validated sampling and analytical methods 42 and 47 using GFFs coated with 1,2-PP and an HPLC with a fluorescence detector. Experimental designs were tailored to evaluate retention and extraction efficiency, detection limits, reliable quantitation limit, thermostability, and storability. Controlled atmospheres of MDI and TDI were not generated.

Instead a liquid and vapor spiking technique were used as an alternative to study the behavior of MDI and TDI once collected in an open-face cassette.

Working standards of MDI-urea derivatives were prepared to avoid polymerization of the isocyanate during evaluations. Briefly, re-crystallized MDI and 1,2-PP were mixed to form white slurry. Purified MDI derivative was obtained following precipitation, filtration, and hexane washing of the slurry. Stock solutions were prepared using DMSO. Subsequent dilutions of the stock were made using ACN to arrive at the working range of MDI. A conversion factor of 0.4339 was calculated by dividing the molecular weight of MDI by the molecular weight of the MDI-urea derivative. The amount of free MDI was then determined by multiplying the weight of MDI derivative by this conversion factor.

Retention efficiency was determined by liquid spiking GFFs with a target amount of MDI using the conversion factor. For example, 9.9µg of MDI derivative was delivered to six GFFs, which was equivalent to 4.3µg of MDI per filter. Subsequently, 20 L of air at 80% relative humidity and 22°C was drawn through the filters to determine retention of the analyte. The average percent recovery of MDI from the filters was found to be 97% using an HPLC and fluorescence detector.

Extraction efficiency for method 47 was determined in a similar approach as above. A target concentration of MDI was delivered to 14 filters; however, no air was drawn through the filter. The extraction efficiency was found to be 96.3%.

To determine TDI retention and extraction efficiencies in sampling method 42, TDI vapor and liquid spikes were employed. Working solutions were also prepared from stock TDI-urea derivatives using ACN. A conversion factor of 0.3479 was calculated to determine free TDI from the derivative standards. When target concentrations of TDI were vapor generated, an

average of 95% retention efficiency of the GFF was determined when 200 L of air with 12% relative humidity was drawn through the filter. However, when the relative humidity was increased to 78%, increasing amounts of TDI was lost with increasing volume of air. Average extraction efficiency for 2,4-TDI was 90.8%.

The retention and extraction efficiencies for OSHA Sampling Method 47 were determined under conditions that facilitated high recoveries of MDI, which do not accurately reflect reaction variables present in the field. For example, preparation of MDI derivatives in a solution environment provided optimal derivatization kinetics, especially without the presence of competitive reactions. Additionally, the solution provided mobility of the MDI-urea standards to evenly distribute across the filter. Previous studies have demonstrated that reagent-coated filters were generally found to yield higher amounts of isocyanates than impingers during side-by-side collection of laboratory-generated MDI atmospheres (Seymour 1987; Coyne 1993; Tucker 1982; Huynh 1992). During field comparisons (e.g., during application of MDI-based polyurethane roof, pouring of MDI at factories and foundries, spray painting) of these samplers, impingers were more likely to give higher results of isocyanates due to the presence of of competitive reactions (Wu 1991; Health. 1984; Andersson 1983; Seymour 1987; Rosenberg 1984). Streicher et al. reasoned that, under laboratory conditions, higher results were attained by filter methods because derivatization kinetics were unchallenged, and therefore unimportant (Streicher, Kennedy, and Lorberau 1994).

Lesage et al. evaluated the efficacy of impinger and filter samplers in a side-by-side comparison during application of polyurethane spray foam (a two-component spray system) inside five single-family homes under construction (Lesage et al. 2007). Personal sampling of applicator exposures (n=13) reported a range of airborne MDI concentrations from 0.07 to 2.05

mg/m^3. Almost two-thirds of the particle mass concentration of the spray foam aerosol was greater than 3.5 microns in diameter, and approximately 20% of the fractions collected were respirable. Results from filter sampling methods indicated a 6% to 40% underestimation of airborne MDI concentrations compared to concentrations determined by the impinger. The authors attributed this difference to the observed particle size distribution.

Isocyanate accessibility to the derivatizing agent is critical when using reagent-coated GFFs, especially in two-component spray applications that consist of an isocyanate and polyol mixture (Booth et al. 2009; Streicher 1994). These products rapidly cure with a half-life of less than two minutes. Micrographs of GFFs containing samples taken during spray applications show minimal contact with the reagent-coated fibers (Bell 1994). As a result, dispersal of the aerosol is negligible, and larger aerosols exhibit an inherent challenge of accessing the reagent (Streicher 1994). Consequently, isocyanates will be lost to competitive reactions within the aerosol mixture and underestimated.

In addition to aerosol particle size and fast cure times, airborne dust or particulate matter may physically hinder isocyanate groups from reacting with the derivatizing agent on the filter media (Karoly 1998; Booth et al. 2009). For instance, MDI may be used as a binder in the manufacturing of engineered wood (e.g., medium density fiberboard). Dust generated in wood mills during this application can impact the collection media and prevent contact of the isocyanate with the derivatizing agent. The isocyanate can be adsorbed onto the surface of the dust or particulate, and undergo a curing reaction before a urea derivative is formed (Booth et al. 2009).

An underestimation of isoycanate exposure puts the worker at a potential risk for a recurring overexposure. In order to preserve the MDI for quantitative analysis, the MDI and

polyol need to be separated, or the curing reaction interrupted (Streicher 1994). Additionally, reagent accessibility needs to be enhanced during fast cure times, generation of large aerosols (>2μm), or both (Streicher 1994).

To improve the performance of filter methods, the filter may be removed from the cassette in the field and desorbed immediately after sampling in a vial containing a solvent miscible with the reactants (Karoly 1998; Streicher et al. 2000). When the filter is desorbed, the extracting solvent will dissolve both the derivatizing reagent and any un-reacted isocyanate, allowing the two to combine in solution and form a stable urea-derivative. Streicher et al. recommend desorbing samples in the field immediately after sampling whenever isocyanates are collected (Streicher et al. 2000). However, OSHA still prescribes filter desorption to occur at the analytical laboratory upon receipt of sample shipment.

Existing literature suggests that a significant difference existed between the results of field- and laboratory-desorbed methods for airborne methylene bisphenyl diisocyanate. Recently, Schaeffer et al. conducted a side-by-side comparison of personal breathing zone samples collected using OSHA Sampling Method 47 and the Wisconsin Occupational Health Laboratory (WOHL) sampling method LC 48, which recommends field desorption. Briefly, the OSHA sampling method 47, and the WOHL sampling method LC 48 are identical in their "upstream" sampling procedures for monomeric MDI, but deviate in post-sampling preparation prior to analysis.

In this study, Schaeffer et al. determined a significant difference between the laboratory desorption technique of OSHA Sampling Method 47 and the field desorption technique of WOHL LC 48. The field-desorbed sampling methodology yielded consistently higher MDI concentrations than the laboratory-desorbed sampling methodology, which suggests that

immediate desorption minimizes isocyanate loss and potential underestimations. Results from the analysis of variance also indicated that the effect of company was significant, meaning that different facility factors and environmental conditions within each company, such as the use of ventilation or humidity level, affected the MDI concentrations; indicating the potential for better mitigation of exposures using the hierarchy of controls.

Since this study was conducted in the field, the true concentration of MDI present during sampling was unknown. Without having a true concentration against which to compare sample results, true accuracy of each method could not be determined, only that a difference between the two methods existed.

While variants of sampling methods for MDI and TDI exist for particular workplace environments(Streicher et al. 2000), companies have greater incentive to use the OSHA methods in order to comply with current regulation. However, in addition to a solution environment that provided optimal derivatization kinetics during validation of GFF methods, aerosols of various particle sizes were not included in the evaluation of measurement accuracy. Since aerosols are a key constituent of a typical exposure scenario involving spray applications of isocyanates, especially MDI, more research is needed to narrow the gap in understanding the effects of particle size on quantitative determinations of MDI.

The purpose of this research is to determine the accuracy of field desorption (FD) and laboratory desorption (LD) techniques while considering aerosol particle size since these methods are commonly used among occupational safety and health professionals even though impingers are the preferred method. To accomplish this goal, an isocyanate aerosol generating system (IAGS) was designed and tested to provide a more realistic evaluation consistent with typical isocyanate exposure scenarios. The aim of this study was to investigate the suitability of

the IAGS in evaluating FD and LD techniques in GFF sampling. The IAGS was eventually used to deliver known amounts of aerosolized isocyanate solutions to determine accuracy of the FD and LD techniques when using GFF samplers.

MATERIALS AND METHODS

Chemicals and Equipment

American Chemistry Society (ACS) grade toluene (CAS No. 108-88-3) suitable for histology and cytology application was acquired from BDH® Chemicals (Radnor, PA).

An EZ-STARTER airbrush set with an atomizing nozzle was purchased from PAASCHE® Airbrush Company (Chicago, IL), which included an air hose with couplings that was connected to a ¼ inch Victor® CGA 346 two-stage gas regulator (Denton, Texas) fitting assembled to a size 300 grade D high pressure breathing air purchased from Airgas (Fort Collins, Colorado).

The Wisconsin Occupational Health Laboratory (WOHL) provided 37 mm, 3-piece, glass fiber filters (GFF) treated with 1, 2- pyridyl piperazine along with desorption vials containing two milliliters (mL) of 90% acetonitrile and 10% dimethyl sulfoxide (90/10 ACN/DMSO).

Description of Isocyanate Delivery System Design

The isocyanate aerosol generating system consists of a KD Scientific 200 Two-Syringe Infusion Pump (KD Scientific, New Hope, Pa.) and an EZ-STARTER airbrush with an atomizing nozzle from PAASCHE® Airbrush Company (Chicago, IL). This airbrush includes an air hose with couplings that connect to an air feed (i.e., grade D high pressure breathing air). A ¼ inch Victor® CGA 346, two-stage gas regulator (Denton, Texas) maintains constant air pressure set at 10 pounds per square inch (psi). Air flows through the air hose and exits the

atomizing nozzle, nebulizing in-coming isocyanate continuously dispensed from 5mL syringes (BD, Franklin Lakes, NJ).

The syringe pump has a menu-driven set up that allows identification of the manufacturer, material, and size of the syringe. This information is used collectively to determine the diameter of the syringe. Subsequently, the pump performs an internal calibration and sets control functions specific to the syringe diameter for continuous dispensed flow rate and volume. Pulsing of solutions is prevented by a micro-stepping motor drive.

The PAASCHE® airbrush is clamped 4" above the syringe pump in a retort stand during each experimental application of isocyanate, which ensures consistency between sample collections. Norprene ® A-60-G, chemically inert tubing, connects the syringes to an airbrush adaptor (item 4, Figure 1-1) in 5" segments. On a separate stand, a 37-millimeter (mm) open-face cassette is positioned 8" from the base, level with the airbrush since the pump has a height of 4". Inside the cassette is a glass-fiber filter (GFF) treated with 1.0 milligrams (mg) of 1-(2-pyridyl) piperazine (1-2PP), as per OSHA Method 47 and 42. The atomizing nozzle is centered slightly inside the plane of the cassette to prevent internal wall losses of the aerosolized solution.

The fundamental assumptions of this research were:

- The IAGS will deliver atomized isocyanates consistent with the reported accuracy and reproducibility of the KD Scientific 200 Two-Syringe Infusion Pump
- Toluene is miscible with isocyanates making a homogenous solution of working standards
- Toluene does not interact with the derivatizating agent, 1,2-PP
- A wide range of isocyanate particle sizes will remain on the filter
- The test aerosols generated are spherical

- The dynamic shape factor (χ) is 1.0
- The aerodynamic diameter and volume equivalent diameter of the test aerosols are equal

Validation of the Isocyanate Delivery System

The reported accuracy and reproducibility of the KD Scientific 200 Two-Syringe Infusion Pump was ±<1% and ±<0.1%, respectively. Quality assurance was independently conducted within the system to ensure sustained delivery of known amounts of MDI and polyol. Five replicates of non-aerosolized water were collected at two connection points, and out of the nozzle (Items A, B, C, Figure 1-1) using various dispense flow rates of the syringe pump. Using a dispense flow rate of 1 ml minute^{-1}, water was collected into a glass beaker for a time of 1 minute.

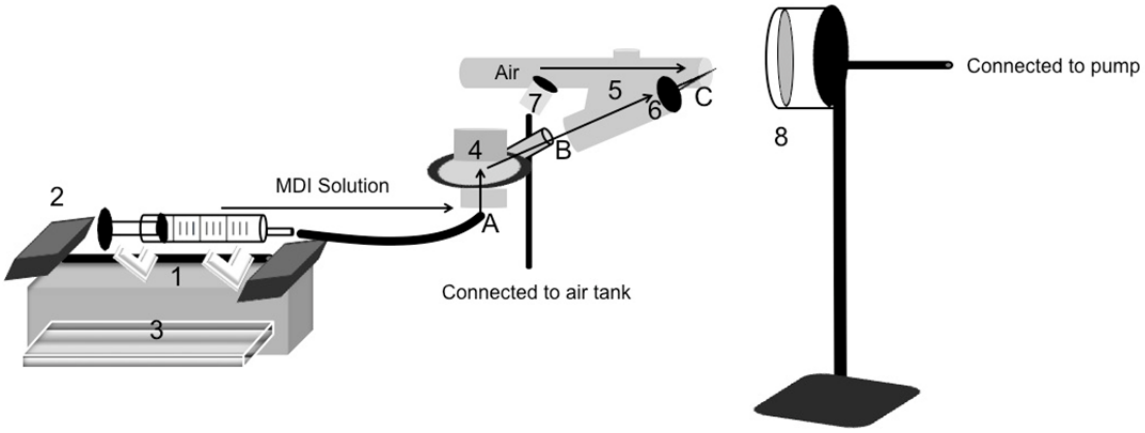

FIGURE 2-3. MDI Delivery System and Sample Collection: 1, the barrel of the syringe rests in the "V" and a retaining arm (not shown) secures the syringe in place; 2, pusher block mechanism that travels along guide rods when drive nut is engaged and depresses syringe plunger at set rate; 3, keypad used to navigate displayed menu features and selection of functions (e.g., syringe manufacturer, size, and diameter, units and rate settings, and directional mode); 4, airbrush adaptor; 5, airbrush; 6, adjustable nozzle; 7, compressed air adaptor; 8, 37-mm open-face cassette; A, connection to the airbrush adaptor; B, connection of adaptor to the airbrush; C, exit out of the nozzle

Generation and Evaluation of Isoycanate Test Aerosols

Preparation of MDI and Polyol Solutions

A local Line-X Company (Loveland, Colorado) provided commercial MDI, PCS 454 A (Eteco, Inc), along with a corresponding material safety data sheet (MSDS). The w/w% of monomeric MDI (CAS No. 101-68-8) reported in the MSDS was less than 50%. Other chemicals reported were a proprietary component (25-45% w/w), and modified MDI (less than 10% w/w). A sample of this commercial MDI was shipped to the WOHL for an independent analysis of the composition. The WOHL determined that PCS 454 A actually contained 0.53 grams of MDI per gram (g) of material using high-performance liquid chromatography (HPLC) methods, which is slightly higher than predicted by the MSDS. Therefore, in order to prepare 10mL of a 1mg/mL MDI stock solution, approximately 18.9 mg of PCS 454 A was weighed out on a Denver Instrument M-series 220D analytical balance and mixed with 10mL of toluene (equation 1). This solvent is readily miscible with isocyanates, and conjectured not to interfere with derivatization of the isocyanate.

(1)

1mg/mL x 10mL = 10mg of MDI;
10mg of MDI from component A requires a
total of 18.9mg based on 1/0.53 x 10mg

From the 1mg/ml MDI stock solution, working toluene solutions containing monomeric MDI concentrations corresponding to 1.3, 2.6, 5.2, and 10.4 micrograms (μg) ml^{-1} were made using equation 2 (and confirmed by HPLC at the WOHL):

(2) $Concentration_1 \times Volume_1 = Concentration_2 \times Volume_2$

This range of MDI concentrations were chosen to evaluate the FD and LD methods at, below, and above the OSHA PEL. Additionally, the MDI working solution of 5.2 μg ml^{-1} is comparable to the concentration of MDI (4.3 μg ml^{-1}) used in the OSHA investigation of retention efficiency. However, solutions were prepared before validation of the flow rate of 1 ml $minute^{-1}$. Accordingly, only a fraction of isocyanate will be delivered to the filter at the adjusted flow rate compared to the original amount proposed. Still using this range of concentrations, the accuracy of FD and LD methods can be evaluated at, below, or above the OSHA PEL through specific iterations of the flow rate of the syringe pump and a sampling pump, which draws air into the filter.

In addition to the PCS 454 A, a bulk sample of commercial PCS 454 B (Eteco, Inc.) was provided. A working solution of PCS 454 B was prepared in the same manner as the 1.3 μg/mL MDI working solution in attempt to preserve the product ratio of MDI to polyol.

Sample Collection

Two small-scale collection studies were conducted using only the FD technique, and both single-, and dual-flow operations of the syringe pump. Since the goal in this phase of the study was to characterize the IAGS and not compare FD and LD, only one desorption method was needed to ensure function, performance, and quality—FD was arbitrarily selected.

The dual-flow operation included two syringes storing isocyanate and polyol separately. This permitted mixing of the co-reactants (i.e., isocyanate and polyol) just before entry into the atomizer in attempt to replicate practical application of fast-reacting isocyanate systems. The goal of this objective was to determine accuracy while assimilating the inherent challenges this reactive mixture poses to observed recoveries of isocyanate. Therefore, each syringe dispensed

known concentrations of isocyanate and polyol that mixed in a plastic Y connector that joins the Norprene ® A-60-G tubing just before entry into the airbrush.

Alternatively, single-flow operation used one syringe, containing a known concentration of isocyanate, and a brass coupling to splice tubing between the syringe and atomizer adaptor. Single flow operation controled for variability caused by reactions between isocyanate and polyol. Accordingly, a baseline result was obtained by spraying isocyanate-only solutions, as well as determination of reaction efficiency between atomized iscocyanate and the derivatizing agent, 1-2PP.

The isocyanate aerosol generating system was set to deliver 0.193 ml min^{-1} of the MDI working solution corresponding to 1.3 μg ml^{-1}, in accordance with the validation described above, to an open-face cassette containing a GFF coated with one milligram (mg) of 1,2-PP. With a short sample time of one minute, a sampling pump was set to draw air at a rate of two liters per minute (L/min) to achieve practical MDI concentrations. A Mine Safety Appliances (MSA) Escort Electronic Laminar Flow (ELF) pump was calibrated at a flow rate of 2 L/min using a Dry Cal DC Lite-DCLT 5K rev 1.08 (Pompton Plains). One sample was collected at a time, and the flow rate was checked post-sampling to ensure they were within ±5% of the original flow rate. The MDI sampling cassettes and desorbing solution were stored in a refrigerator before use as prescribed by OSHA method 47 and WOHL method LC48.

Theoretically, a mass of 0.251 μg is delivered at the end of one minute in the single-flow operation. Accordingly, a theoretical concentration can be calculated based on total volume (i.e., 2 L or 0.002 m^3), which is approximately 125μg/ m^3. This concentration is almost half of the OSHA PEL.

Similarly, the dual-flow operation was set up to deliver MDI and polyol at a rate of 0.193 ml min^{-1}. Without a known rate of isocyanate consumption in the reaction with a polyol, theoretical calculations of delivered isocyanate are not possible. Therefore, a baseline was collected to determine the amount of unreacted MDI monomer in the dual-flow operation and compared against MDI-only solutions sprayed.

After the sampling cassettes were removed from the apparatus, the FD filter was removed using forceps and placed into a glass vial containing 2 mL of 90/10 ACN/DMSO desorbing solution. The researcher agitated the vial gently to ensure that the entire filter was saturated with desorbing solution. The vial was sealed with the cap provided by WOHL and wrapped with parafilm around the top of the vial to prevent leaking.

Accuracy of the FD and LD techniques were evaluated based on comparing HPLC-quantitated results from the WOHL, an American Industrial Hygiene Association (AIHA) accredited laboratory, to the theoretical results calculated from concentration of MDI solution, flow rate of syringe pump, and sampling time. By comparing observed to theoretical, accuracy and consistency of each diisocyanate field sampling method can be analyzed.

Toluene Interaction

The NIOSH Method 5521, an impinger method, uses a solution of 1,2-PP in toluene to capture monomeric MDI and TDI. Impinger methods have been shown to enhance derivatization kinetics of large MDI/polyol aerosols in the field. Therefore, no interaction was anticipated between toluene and 1,2-PP; however, investigations were conducted to ensure derivatization of the isocyanates was not affected by the presence of residual toluene.

Working solutions of MDI were prepared using a fixed amount of MDI and varying the amount of toluene. This is different than how the MDI working solutions were prepared since

volumes were adjusted for both MDI and toulene. In equation 2, both the amount of MDI and toluene are varied to achieve the target concentration. Briefly, three different 5 ml aliquots were used to prepare dilutions corresponding to 1:2, 1:3, and 1:4 of MDI to toluene. Each aliquot was mixed in an Erlenmeyer flask with 5, 10, and 15 ml of toluene. Additionally, an undiluted 10.4 $\mu g\ ml^{-1}$ MDI solution was used as a reference.

To evaluate the effect of toluene only, MDI-only solutions were sprayed as described in the sampling procedures using three different dilutions of the MDI solution containing 10.4 μg per ml of toluene. All GFF samples were FD. Five samples were collected for each solution to detect a trend of decreasing MDI with results consistent with each dilution factor. In other words, each dilution should decrease the amount of MDI by half.

Particle size

A Grimm Dust Monitor Model 1.108 (Grimm Technologies, Inc., Douglasville, GA) was used to determine particle size distribution of the IAGS. Gravimetric analysis of a 47-mm PTFE filter is used to verify the reported mass of the aerosol. The Grimm Dust Monitor 1.108 measures particle number concentration by size from 0.3μm to 20μm in 15 size distribution channels. It was assumed that this range of particle sizes would be sufficient to capture the particle size distribution of the IAGS. The max number (particles cm^{-3}) and mass ($mg\ m^{-3}$) concentrations were 2000 and 100, respectively. In order to accurately determine the particle size of isocyanates, toluene-only solutions were also analyzed.

To convert the number output of the Grimm to a particle mass concentration, the particle density (ρ_p) of each working solution was calculated since mass percent of both components changed per mixture (equation 3). The density of toluene and isocyanate are 0.8669 and 1.23 g

cm^{-3}. Particle mass concentrations ($\mu g/m^3$) at each cut-point were calculated by multiplying counts/l (particles cm^{-3}) by the mass of each particle (equation 4).

(3)

$$\text{Density}_{\text{mixture}} = 1/(\%_{\text{mdi}} / \text{density}_{\text{mdi}}) + (\%_{\text{toluene}} / \text{density}_{\text{toluene}})$$

(4)

$$dM_i = \pi/6\ (\rho_p D^3_{p,i})\ dN_i$$ (Peters, Ott, and O'Shaughnessy 2006)

where ρ_p represented particle density, and $D^3_{p,i}$ and dN_i represent the midpoint aerodynamic diameter and number concentration measured in the *i*-th channel of the Grimm (Peters, Ott, and O'Shaughnessy 2006).

Wet MDI aerosols from the IAGS were generated in a chemical laboratory hood in direct line with the Grimm Dust Monitor 1.108. Ambient air inside the chemical hood was analyzed before test aerosols were generated to ensure no interference occurred. The IAGS was run for one minute, and particle size distributions were reported every six seconds. Five replicates per working solution were collected over 1 min. each, producing more than 50 measurements reported for the various size distribution channels.

The count median diameter (CMD), or diameter of a particle where half the count of the aerosol was contained in smaller diameter particles and half in larger (O'Callaghan and Barry 1997), was calculated for each working solution of MDI. The arithmetic average of particles cm^{-3} measured in each size interval of the distribution channel was calculated and tabulated in ascending order of particle size. The summation of total particles cm^{-3} allowed computation of the cumulative percent of the grouped data. Interpolating between two data points containing the 50% point (median), the CMD was determined. These iterative calculations were also used to determine the MMD, the 50% point separating the aerosol into two halves based on mass. The geometric standard deviation (GSD), which is a dimensionless number equal to or greater than 1.0 depending on the range of sizes of particles making up the aerosol, was also determined by

interpolation of the cumulative percent of the grouped data. For lognormal distribution, a ratio between the cumulative count of 84% and 50% (CMD) represented the GSD (Hinds 1999).

To determine the dry particle size, IAGS aerosols were generated in a $1m^3$ chamber. Alternatively, an APS and SMPS were used to increase resolution since smaller particles were anticipated. Vacuum oil and ethyl alcohol were used as surrogates instead for this evaluation since MDI and toluene were not compatible with the components of the chamber or the particle sizing instrumentation. Vacuum oil was selected since it had a similar vapor pressure as MDI. A solution of vacuum oil was prepared with the same mass concentration as MDI working solution 5, which contained 11.0 µg of MDI per ml of toluene. Three replicates of aerosols were generated over 1 min. each. With the introduction of sheath air, aerosols were anticipated to dry rapidly. IAGS aerosol particle sizes consisting of vacuum oil were measured from 0.001µm to 5.8 µm. The data at each bin size were averaged, and the sum of the mass concentration was used to calculate the cumulative percent.

RESULTS AND DISCUSSION

Validation of the Isocyanate Delivery System

Accuracy of the dual-flow operation was based on the capability of the system to deliver a mass of water at a specified flow rate that was equivalent to the density of water (1g/mL). A Denver Instrument M-series 220D analytical balance (Arvada, CO) was used according to the United States Pharmacopoeia (USP) minimal sample weight for reliable measurements (A&D Company 1998). Accordingly, a minimum sample weight of 0.01mg was derived for this M-series 220D analytical balance at a capacity of 31 grams.

A dispense flow rate of 1 ml $minute^{-1}$ and collection time of 1 minute, corresponded to a mass equal to 2g. The average and standard deviation were calculated to determine reproducibility (Table 2-1). The percent error associated with comparing the experimental results

with the theoretical quantity indicated accurate recoveries at each point within the system. Most importantly, collection of water out of the nozzle yielded a percent error of only 0.68, which is less than 1% as reported by the manufacturer.

TABLE 2-1. Experimental results of syringe performance during dual-flow operation at a dispense flow rate of 1 mL minute^{-1}.

Item	Average Net Increase Of Mass (g)	Standard Deviation (g)	Percent Error (%)
A	2.0024	0.02087	0.12
B	2.0094	0.00875	0.47
C	2.0137	0.01489	0.68

Subsequently, atomized water was collected onto tared GFFs treated with 1.0 milligrams (mg) of 1-(2-pyridyl) piperazine (1-2PP) contained in a 37-millimeter (mm) cassette; dispense flow rate of the syringe pump was 1mL minute^{-1}. An aerosolized sample was collected for a time of 1 minute to determine the size of the spray pattern in an open-face cassette. While no losses were observed to the inside walls of the cassette, the filter was overloaded with a volume of 1 and 2 mL of water during single-, and dual-flow operations.

Accordingly, different dispense rates using single-, and dual-flow operations were evaluated over a collection time of 1 min. to determine an appropriate volume that did not overload the filter. Anecdotally, by adjusting the flow rate of the pump, target amounts of MDI could be achieved using a standard solution of 1μg/ml. A flow rate of 0.160 ml minute^{-1} was examined, which would deliver 0.160 μg per syringe. However, after collecting aerosolized water samples from the dual-flow operation of the IAGS, the average results were 241 mg, which was approximately 80 mg lower than the expected 320 mg. Initially, evaporation was not considered, so the flow rate was adjusted to 0.193 ml minute^{-1} to achieve 0.320 μg on the filter. This produced a total volume of 0.386 ml, which did not exceed the capacity of the filter.

Therefore, this volume was designated as the uppermost limit during each experiment. Additionally, a lower limit of 0.193 mL was included to coincide with spraying diisocyanate-only solutions. Five replicates were collected at these adjusted flow rates and weighed immediately. With an experimental value of <1% error out of the nozzle (Table 2-2 and 2-3) when using non-aerosolized water, the authors felt confident in the apparatus to deliver accurate and reproducible amounts of isocyanate at a range of concentrations ($\mu g/m^3$) below and above the OSHA Permissible Exposure Limit (PEL), which is representative of workplace exposures.

TABLE 2-2. Experimental results of syringe performance during single-flow operation at a dispense flow rate of 0.193 mL minute^{-1}.

Item	Average Net Increase of Mass (g)	Standard Deviation (g)	Percent Error (%)
A	0.1929	0.003	0.05
B	0.1914	0.001	0.82
C	0.1946	0.002	0.82

TABLE 2-3. Experimental results of syringe performance during dual-flow operation at a dispense flow rate of 0.193 mL minute^{-1}.

Item	Average Net Increase of Mass (g)	Standard Deviation	Percent Error (%)
A	0.3863	0.002	0.07
B	0.3862	0.002	0.05
C	0.3871	0.003	0.28

Evaporation was not initially considered during validation of the IAGS using aerosolized water samples. The "calibrated" flow rate of 0.193 ml minute^{-1} in order to achieve 0.160 μg from each syringe was re-examined. Consequently, the net increase in filter weight from atomized water was not included in the delivery system validation. The amount of isocyanate delivered was assumed to be consistent with the flow rate of the syringe pump and concentration of the isocyanate working solution. Briefly, upon atomization of the water, the rate of evaporation is increased since a change of volume over time (dV/dt) is proportional to surface area (S) of the

droplet. Therefore, weighing net increase in filter weight would show an underperformance of the system based on comparisons of observed to theoretical results derived from the dispensed flow rate, time, and density of water. However, since isocyanates have a much lower vapor pressure compared to water (Table 2-4), evaporation of isocyanates was not a concern. Additionally, the solvent toluene, which also has a high vapor pressure (Table 2-4) compared to MDI and TDI, was used to prepare working solutions of monomeric MDI. This solvent was anticipated not to interact with the 1,2-PP reagent upon deposition on the filter, and assumed to continually evaporate during and after sample collection.

TABLE 2-4. Vapor pressures for water, MDI, and TDI.

Chemical Substance	Vapor Pressure (77 °F)
Water	314.1 mmHg
Toluene	28 mmHg
MDI	$5x10^{-6}$ mmHg
TDI	$1x\ 10^{-2}$ mmHg

Sample Collection

In this pilot study, a total of 10 samples were collected from test atmospheres generated from the IAGS. These test samples were used to evaluate the proficiency of the system to effectively generate a test atmosphere with reproducibility between replicates during each flow operation of the pump. Results from the single-flow operation of the syringe pump are shown in Table 2-5. Theoretically, the IAGS delivered 251ng of MDI to the GFF, based on concentration of the solution (1.3 μg ml^{-1}), flow rate of the syringe pump (0.193 ml $min.^{-1}$), and sample time (1 min.). The flow rate of the personal pump connected to the cassette was only considered during calculations to determine concentration (not reported in this study).

TABLE 2-5. Descriptive statistics of field-desorbed MDI samples collected from the IAGS using single-flow operation.

	Single-flow operation	Dual-flow operation
Desorption method	FD	FD
Flow Rate (ml/min)	0.193	0.193
Component Sprayed (1.3µg/ml)	MDI only	MDI and polyol
Theoretical Amount of MDI (ng)	251	NA
Observed Amount of MDI (ng)	224 (n=5)	178 (n=5)
Standard Deviation of Observed (ng)	18.1	44.4
Coefficient of Variation (%)	8.1	25
Percent Recovery Average (observed/theoretical)(%)	89.2	NA
Percent Recovery Standard Deviation (%)	7.5	NA
Percent Error (comparing single-flow to dual-flow operation)	NA	21

Quantitative determinations of MDI contained on these five FD GFFs were made using an HPLC at the WOHL. The average of these five replicates was 224ng with a standard deviation of 18.1ng. When compared to the theoretical mass, an 89.2 % recovery (± 7.5%) was determined for FD samples collected from the IAGS. This underestimation of MDI was most likely due to minimal contact between the isocyanate and the derivatizing agent.

The OSHA demonstrated a 97.4% (± 1.1%) recovery from liquid spiking 9.9µg MDI-urea derivative (4.3µg free MDI); however, the standard deviation was ±48ng when calculated in a unit of mass. The individual percent recoveries are shown in Table 2-6. The mass recovered from each sample collected was calculated using the target amount of 4.3µg free MDI and each percent recovery. Subsequently, an average mass of 4.19 µg and standard deviation of 48ng were determined.

TABLE 2-6. Retention efficiency of liquid spiking 9.9μg MDI-urea derivative (4.3μg free MDI) onto six individual GFFs.

Percent recovery (%) (±1 SD)	Mass recovery (μg) (±SD)
95.7 (±1.1)	4.11 (± 0.048)
96.8 (±1.1)	4.16 (± 0.048)
97.2 (±1.1)	4.18 (± 0.048)
98.4 (±1.1)	4.23 (± 0.048)
98.8 (±1.1)	4.25 (± 0.048)
97.5 (±1.1)	4.19 (± 0.048)

A lower percent recovery was expected from an aerosol-spiked filter compared to a liquid-spiked filter. The IAGS atomized free MDI as opposed to a stable derivative used in the OSHA experiment. Free MDI is capable of competitively reacting with the moisture in the air, and other agents present with active hydrogens, in addition to 1,2-PP. Toluene that impacted the filter may have facilitated a nominal dispersion of isocyanate across the filter; however, the solution environment in the liquid spike provided an unmatched mobility of isocyanate. Consequently, the working area of the filter was maximized, preventing agglomeration or clustering of isocyanates.

Since liquid aerosol particles adhere when they impact a surface, interparticle adhesion likely formed an aggregate of MDI. Formation of an isocyanate layer on a GFF is likely to occur during practical application in the field. As a consequence, any MDI not in direct contact with 1,2-PP may be competitively excluded.

While the IAGS exhibited a 7-fold increase in variability describing percent recovery compared to the liquid spike of OSHA, the variability in mass was a more accurate indicator of performance. The OSHA used a target mass that was approximately an order of magnitude higher, reducing sensitivity to the influence of deviations from the mean as compared to the

current data set, especially in terms of percent. Therefore, a comparison between percent standard deviations from each experiment was misleading.

The small variability of the IAGS was expected due to the accuracy and precision of the syringe pump used; however, this variability was still greater than <0.1% as reported by KD Scientific. This disparity in precision most likely reflects difference in reaction kinetics with 1,2-PP influenced by either particle size, or environmental conditions (e.g., relative humidity, other airborne agents present in the laboratory), or both.

Five replicates were also collected from the dual-flow operation at 0.193 mL minute^{-1} and these results are also presented in Table 2-5. A theoretical mass was not calculated since MDI and polyol were mixed and then aerosolized. Determining the percent yield from polymerization at these low concentrations was outside the scope of this study. Therefore, samples were collected to establish a baseline and compare it to the analytical results from the single-flow operation. An average of 178ng was calculated from the five replicates with a standard deviation of 44.4ng. Comparing this average to the theoretical amount of MDI sprayed, a percent recovery of 70.9% was determined. Percent error between the single-, and dual-flow operation was approximately 21%.

Based on recovered MDI from the dual-flow operation, isocyanate consumption was evident upon mixing with the polyol. The dual-flow operation yielded an even greater underestimation in GFF sampling of MDI compared to the single-flow operation. Since an underestimation was preliminarily determined in isocyanate-only samples, the GFF method may be a contributing factor related to derivatization kinetics. Therefore, the underestimation in the dual-flow operation cannot be attributed exclusively to consumption of isocyanates through polymerization. Rather, both polymerization and limited contact with the derivatizing agent

were considered likely candidates accounting for underestimation of MDI in the dual-flow operation.

Toluene Interaction Results

A new flow rate for the single-flow operation of the syringe pump was validated and used during this experiment. Briefly, during the validation process, the filter was oversaturated after spraying 1ml of water. Accordingly, the flow rate of the syringe pumped was reduced to deliver a manageable volume to the filter. Since MDI solutions were already prepared in the range previously described, an MSA ELF pump was calibrated to 2 L/min in order to achieve a practical range of MDI concentrations in parts per billion (ppb). Theoretically, 250.1 ng was delivered to a GFF during single-flow operation, which is the equivalent to 15 ppb (equation 5).

(5) $mg\ m^{-3} = ppm\ (MW) / 24.45\ (P_o/P_m)\ (T_m/T_o)$

	$mg\ m^{-3}$ =	Mass concentration based on HPLC results and volume of air collected
	ppm=	parts per million
	MW=	Molecular weight of MDI
where :	P_o=	Normal pressure
	P_m=	Measured pressure
	T_o=	Normal temperature
	T_m=	Measured temperature

To maintain the logarithmic concentration scale of MDI, the flow rate was adjusted to 0.380 ml $min.^{-1}$. This adjusted flow rate allowed a stepwise increase in the amount of MDI from the starting value of 250.1ng while avoiding the upper volume limit of 0.386 ml. Therefore, a solution of 11 μg/ml sprayed at a rate of 0.380 ml $min.^{-1}$ generated a theoretical amount 4.2 μg, or 204 ppb.

To validate a dispense flow rate of 0.380 ml $min.^{-1}$, five replicates of non-aerosolized water were collected for a time of 1 minute. These settings corresponded to a mass equal to

0.380 g. All five replicates were averaged, and these results are illustrated below (Table 2-7). Referring back to Figure 1-1, an experimental value of <1% error was determined at items A-C of the IAGS. Accordingly, the authors felt confident using this flow rate in this and future experiments.

TABLE 2-7. Validation of a dispense flow rate of 0.380 ml min. $^{-1}$

Item	Average Net Increase of Mass (g)	Standard Deviation (g)	Percent Error (%)
A	0.378	0.003	0.5
B	0.381	0.003	0.2
C	0.378	0.003	0.5

To evaluate if an interaction exists between toluene and 1,2-PP, a starting solution of 10.4 μg/ml of MDI was diluted three different times. Increasing volumes of toluene were used to prepare a geometric progression of MDI concentrations. A total of four concentrations of MDI were used in this experiment, ranging from 1.3 to 10.4 μg/ml.

The dilution factor and corresponding volume are shown in Table 2-8. Individual dilutions were prepared to avoid propagation of error associated with serial dilutions. Three individual samples were collected for 1min. per dilution of MDI, beginning with the lowest concentration. Each successive dilution was assigned a numeric index, ranging from 1 to 4, to categorically represent the data from highest to lowest concentrations of MDI (e.g., concentration 1 represents the working solution 10.4 μg/ml).

A total of 12 FD-only samples were collected in this evaluation, and analyzed by HPLC at the WOHL. Results were averaged for each dilution (Table 2-8). The mean values of MDI mass for concentrations 1-4 were as follows: 3.2 μg (range, 3.0 to 3.5 μg); 1.4 μg (range, 1.3 to 1.6 μg); 0.86 μg (range, 0.84 to 0.93 μg); and, 0.53 μg (range, 0.49 to 0.55 μg). A relatively small standard deviation (SD) was noted in each range of the data (Table 2-8).

TABLE 2-8. Toluene dilutions of technical grade MDI

Concentration Level	Volume (ml) of MDI solution containing 10.4μg	Dilution factor	Volume (ml) of toluene	Theoretical amount of MDI (μg)	Experimental amount of MDI (μg) (± 1 SD)
1	5ml	na	0ml	4.0	3.2 (±0.26)
2	5ml	1:2	5ml	2.0	1.4 (±0.15)
3	5ml	1:3	10ml	1.0	0.86 (±0.06)
4	5ml	1:4	15ml	0.5	0.53 (±0.03)

NA= Not applicable since this was the starting concentration

As shown in Table 2-8 analytical results underestimate the amount of MDI compared to the theoretical amount, except in concentration 4. A comparison between analytical and theoretical was included to verify accuracy of the dilution; however, based on these results, accuracy remained uncertain. For example, concentration 2 was approximately 5.2 μg/ml, which would have delivered a nominal 2.0 μg/ml of MDI. The percent recovery of MDI in concentration 2 was 70%. This uncertainty was also compounded by the sample size and common ratio between dilutions. Theoretically, the common ratio of this dilution sequence is 2. The observed ratios between concentrations 4 and 3, and each successive dilutions were 2.3, 1.6, and 1.6, respectively.

While this large of an underestimation was not unexpected based on the 89.2 percent recovery previously described, further analysis was required. New MDI working solutions were needed to repeat this experiment. The original sample set had already been dedicated to aim 2. Pure MDI (4,4'-methylenbis(phenylisocyanate) was purchased from Sigma-Aldrich (St. Louis, MO). A new range of working solutions corresponding to 1.4, 2.7, 5.3, and 11.0 μg/ml were prepared from and confirmed by HPLC analysis at the WOHL. The MDI solution of 11.0 μg/ml served as the starting solution, and was diluted in the same progressive sequence previously

described. Working solutions from highest to lowest concentrations of MDI were numerically represented beginning with 5 and ending at 8.

A total of 20 FD-only samples were collected in this evaluation, and again analyzed by HPLC at the WOHL. Results were averaged for each dilution (Table 2-9). The mean value of MDI mass for concentrations 5-8 was as follows: 3.1 μg (range, 2.5 to 3.3 μg); 1.7 μg (range, 1.6 to 1.7 μg); 1.2 μg (range, 1.1 to 1.2 μg); and, 0.79 μg (range, 0.72 to 0.83 μg). Again, a relatively small standard deviation (SD) was noted in each range of the data (Table 2-9).

TABLE 2-9. Toluene dilutions of pure grade MDI

Concentration Level	Volume (ml) of MDI solution containing 11.0μg	Dilution factor	Volume (ml) of toluene	Theoretical amount of MDI (μg)	Experimental amount of MDI (μg) (± 1 SD)
5	5ml	na	0ml	4.20 μg	3.1 (±0.35)
6	5ml	1:2	5ml	2.10 μg	1.7 (±0.05)
7	5ml	1:3	10ml	1.05 μg	1.2 (±0.05)
8	5ml	1:4	15ml	0.525 μg	0.79 (±0.05)

NA= Not applicable since this was the starting concentration

Theoretical and experimental results are also shown in Table 2-9. A decreasing trend of MDI mass was observed. The common ratios between successive dilutions (from highest to lowest) were 1.8, 1.4, and 1.5, showing a similar pattern as the previous data set.

Consistency between data sets indicates that toluene does not interact with 1,2-PP. Two different concentrations reproduced similar ratios using different lot numbers of GFF cassettes. While a common ratio of 2 was not produced, volumetric glassware was not used to prepare dilutions, which are calibrated to contain a precise volume. Since graduated cylinders were used, precision between working solutions was compromised. However, the same technique and equipment was repeated to prepare each dilution in each trial. Accordingly, the precision in preparing the working solutions was equal throughout the experiment. The concomitant decrease

in the amount of MDI (i.e., both technical and pure grades) with each successive dilution is shown in Figure 2-4.

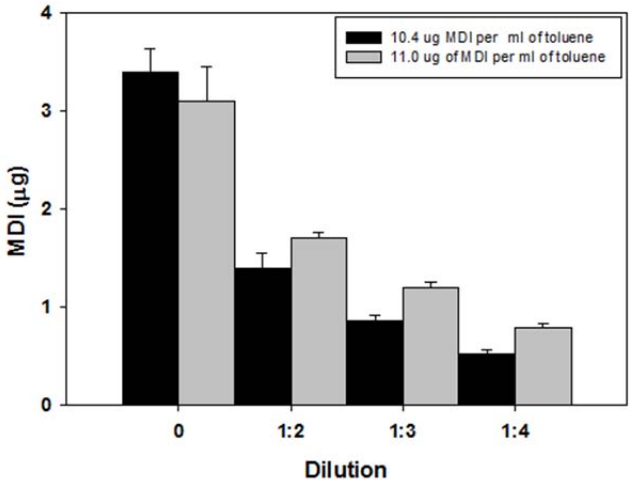

FIGURE 2-4. Successive dilutions and concomitant decrease in the amount of MDI.

In both trials, analytical results either underestimated or overestimated the theoretical amounts of MDI. The overestimation of MDI in concentrations 4, 7, and 8 was attributed to the lack of precision in preparing dilutions. A larger amount of MDI may have been measured inadvertently using a graduated cylinder instead of a volumetric flask. Transferability of analyte (or lack thereof) during dilutions may also explain the observed underestimations of MDI in concentrations 2, 3, and 6; however, concentrations 4 (10.4 µg/ml) and 5 (11.0 µg/ml) were quantitated by HPLC before application providing an accurate comparison between theoretical and experimental.

An uneven distribution of 1, 2-PP on GFFs may also cause an underestimation of isoycanate due to breakthrough from a limited capacity to stabilize the analyte (Tucker 2007). In 2007, Tucker investigated reagent distributions on commercially available filters by analyzing 5-mm punches from a variety of GFFs (Tucker 2007). A range of 309 to 576 µg of 1,2-PP per filter with a standard deviation of 85.9 µg was determined in the analysis of eight filters in the same

lot. Analysis of twenty-one 5-mm punches of a GFF yielded a relative standard deviation of 30.8%. The largest quantity of 1,2-PP was 28.0 μg, which was found in the center of the filter while the smallest amount, 9.3 μg, was at the perimeter of the filter.

Hydrogen bonding of 1,2-PP may decrease mobility of the reagent across the surface of a GFF. Two common techniques for coating GFFs include total immersion of a filter in a reagent solution, or syringe application of reagent solution 1cm above the center of filter. The molecular structure of 1,2-PP contains three nitrogen atoms, accounting for 26% of the molecular weight. Hydrogen bonding is likely to occur between similar molecules, as well as hydroxyl groups on the surface of a GFF.

While the aim of this study was to characterize the IAGS and not evaluate the FD method, these results indicate that desorption of filters in the field may significantly underestimate the true concentration of MDI. Therefore, more research is needed to evaluate FD and LD methods using the IAGS to determine which method is more accurate.

Particle Size Results

The IAGS was expected to generate isoycanate aerosols with a wide range of particle sizes influenced by the density and viscosity of the solution. Other influencing factors affecting the size distribution, which were not tested, included vapor pressure, surface tension of solution-air interface, nozzle design and pressure, and environmental conditions (e.g., temperature and relative humidity).

Pure-grade MDI solutions that were prepared to evaluate the interaction of toluene and 1,2-PP was also used in this investigation as the technical grade MDI was expired. The density of each mixture is summarized in Table 2-10. The density of toluene and isocyanate are 0.8669 and 1.23 g cm^{-3}. The mixture containing 1.4 μg of MDI per ml of toluene was used at both flow rates,

representing working solution 1 and 2. The density of these working solutions was slightly greater than the density of toluene. As the mass percent of MDI increased and toluene decreased, the density of working solutions 3 through 5 increased.

TABLE 2-10. Density of MDI working solutions

MDI/Toluene Mixture (Working Solutions)	Density ($g\ cm^{-3}$)
1.4 μg MDI/ml of toluene (Working solution 1 & 2)	0.867372
2.7 μg MDI/ml of toluene (Working solution 3)	0.867844
5.3 μg MDI/ml of toluene (Working solution 4)	0.868788
11.0 μg MDI/ml of toluene (Working solution 5)	0.870676

As shown in Figure 2-5, distribution of IAGS aerosol data was approximately normal. To evaluate the distribution of the data, a plot of the quantiles was generated. Count data were used from the toluene-only solution dispensed at a flow rate of 0.193 ml $min.^{-1}$. Data were logarithmically transformed since aerosol particle size distributions were skewed to the right, indicated by a long tail at the larger particle sizes.

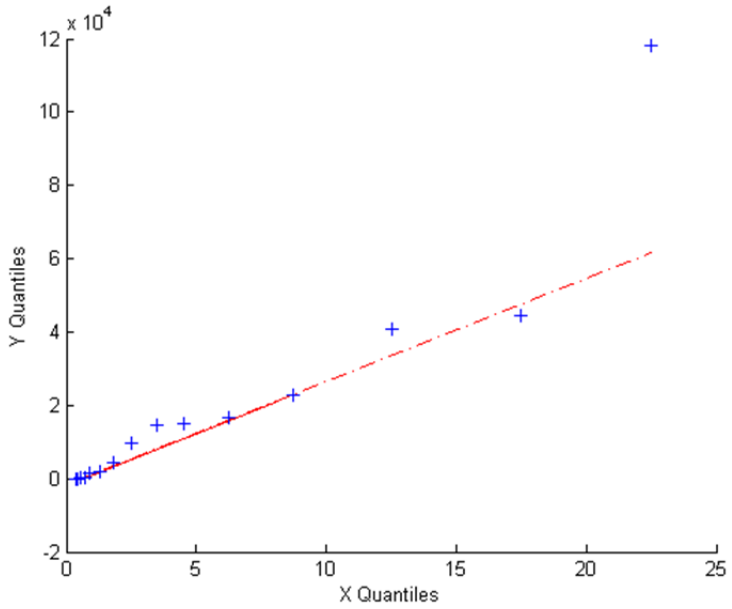

FIGURE 2-5. Q-Q Plot of toluene data (at a flow rate of 0.193 ml min.$^{-1}$) log-transformed.

Figure 2-6 presents a summary of particle size distribution from the IAGS in a number histogram. The number of particles cm^{-3} was normalized to the size range collected in each channel. This interval-normalized number frequency plot presents the $dN/dlogD_p$ (particle cm $^{-3}$) on the y-axis and the particle size (log scale) on the x-axis. The width of each bar corresponds to the width of the measurement bin. MDI aerosols generated by the IAGS exhibited a unimodal size distribution present under 1 µm. The number concentrations between toluene solutions (i.e., blank solutions delivered at 0.193 ml min.$^{-1}$ and 0.380 ml min.$^{-1}$) and MDI working solutions 1-5 were comparable and remained moderately consistent. As shown in Figure 2-6, the high number concentrations observed in the measurement bins under 1µm account for nearly 85% of the total number concentration measured. Descriptive statistics are shown in Table 2-11.

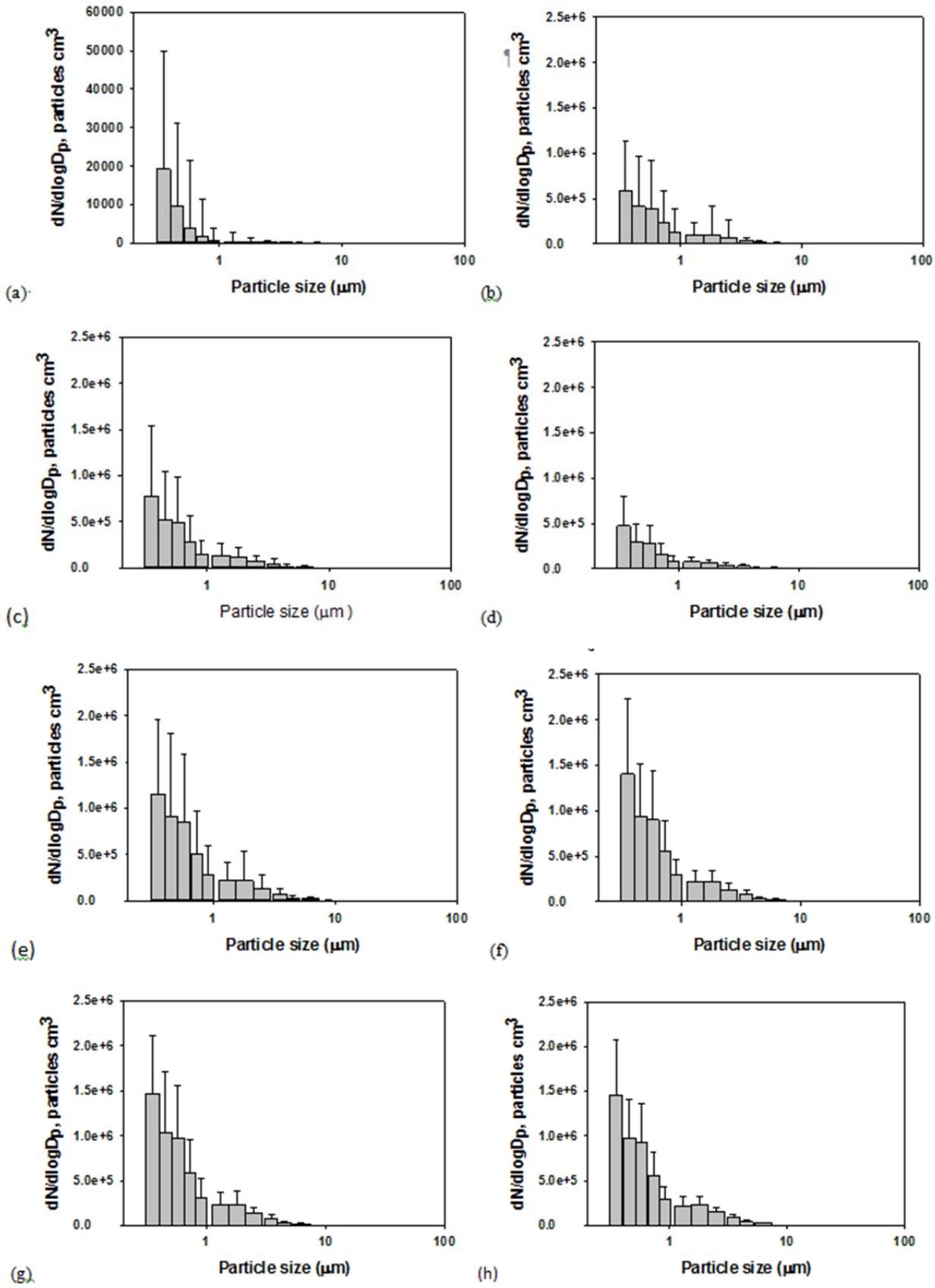
dN/dlogDp, particles cm3
Particle size (µm)
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

FIGURE 2-6. Distribution of particle number concentration ($dN/dlogD_p$) normalized by size measured (error bars represent 1 SD): (a), background air in chemical hood; (b), toluene at a flow rate of 0.193 ml $min.^{-1}$; (c), toluene at a flow rate of 0.380 ml $min.^{-1}$; (d), MDI working solution 1; (e), MDI working solution 2; (f), MDI working solution 3; (g), MDI working solution 4; (h), MDI working solution 5

Table 2-11. Summary of MDI particle count distribution and statistics

Statistical parameter	Toluene 1	Toluene 2	MDI 1	MDI 2	MDI 3	MDI 4	MDI 5
CMD, μm	0.41	0.40	0.38	0.42	0.40	0.40	0.40
GSD	2.18	2.19	2.16	2.14	2.17	2.24	2.14
Total concentration, particles cm^{-3}	288,547	370,576	216,072	603,683	677,316	718,050	692,052

The CMD and corresponding geometric standard deviation (GSD) remained approximately constant across all solutions. Approximately 50% of the counts per cm^{-3} were contained between the two lower size bins or distribution channels of the Grimm. Therefore, a discrepancy may exist in the classification of these aerosols at smaller bin sizes due to size resolution, which was evaluated over 15 channels only. For example, the Grimm Dust Monitor Model 1.109 (Grimm Technologies, Inc., Douglasville, GA) contains 31 channels while the Aerosol Particle Sizer (APS) model 3321 (TSI, Inc., St. Paul, MN, USA) has 52 channels (Peters, Ott, and O'Shaughnessy 2006). More than 40% of the counts were observed in the 0.35μm distribution channel, which suggests an array of aerosols smaller than the 0.35 μm may have been generated and consequently undetected. For this study, the Grimm Dust Monitor Model 1.108 (Grimm Technologies, Inc., Douglasville, GA) was sufficient to characterize the IAGS aerosol size distribution; however, to acquire a more accurate representation of the aerosol particle size distribution, an APS and a Scanning Mobility Particle Sizer (TSI, Inc., St. Paul, MN,

USA) can be used in combination, allowing a broader size range to be measured (e.g., 2.5 nm to 20μm).

The mass concentration by size is presented in Figure 2-7. Like in Figure 2-6, this is an interval-normalized mass frequency plot with $dM/dlogD_p$ ($\mu g/m^3$) on the y-axis and particle size (log scale) on the x-axis. As shown in Figure 2-5, the mass concentration has shifted to larger sizes, which also exhibited a bimodal size distribution with one mode under 6.25 μm and a second mode above 20 μm. This much larger mode was not expected. When compared to background air in the chemical hood, the response at the 22.5 μm cutpoint was almost 5 orders of magnitude greater when each solution was sprayed. Descriptive statistics for these data are shown in Table 2-12.

The mass median diameter (MMD) was expected to increase with higher concentrations of MDI, based on moment averages. The mass of a particle is proportional to d^3, based on the relationship between volume and density. Accordingly, masses were calculated for each particle size cutpoint associated with the 15 distribution channels contained in the Grimm. Since the density of each working solution increased with percent mass of MDI, the mass of each particle size increased as well. For example, the theoretical mass of a 1.3 μm droplet produced from MDI working solution 1 and 2 was 9.98×10^{-7} μg. The mass of the same size droplet produced from MDI working solution 5 was 1.0×10^{-6} μg, which was an order of magnitude greater—theoretically increasing the MMD slightly with each increasing mass concentration of MDI.

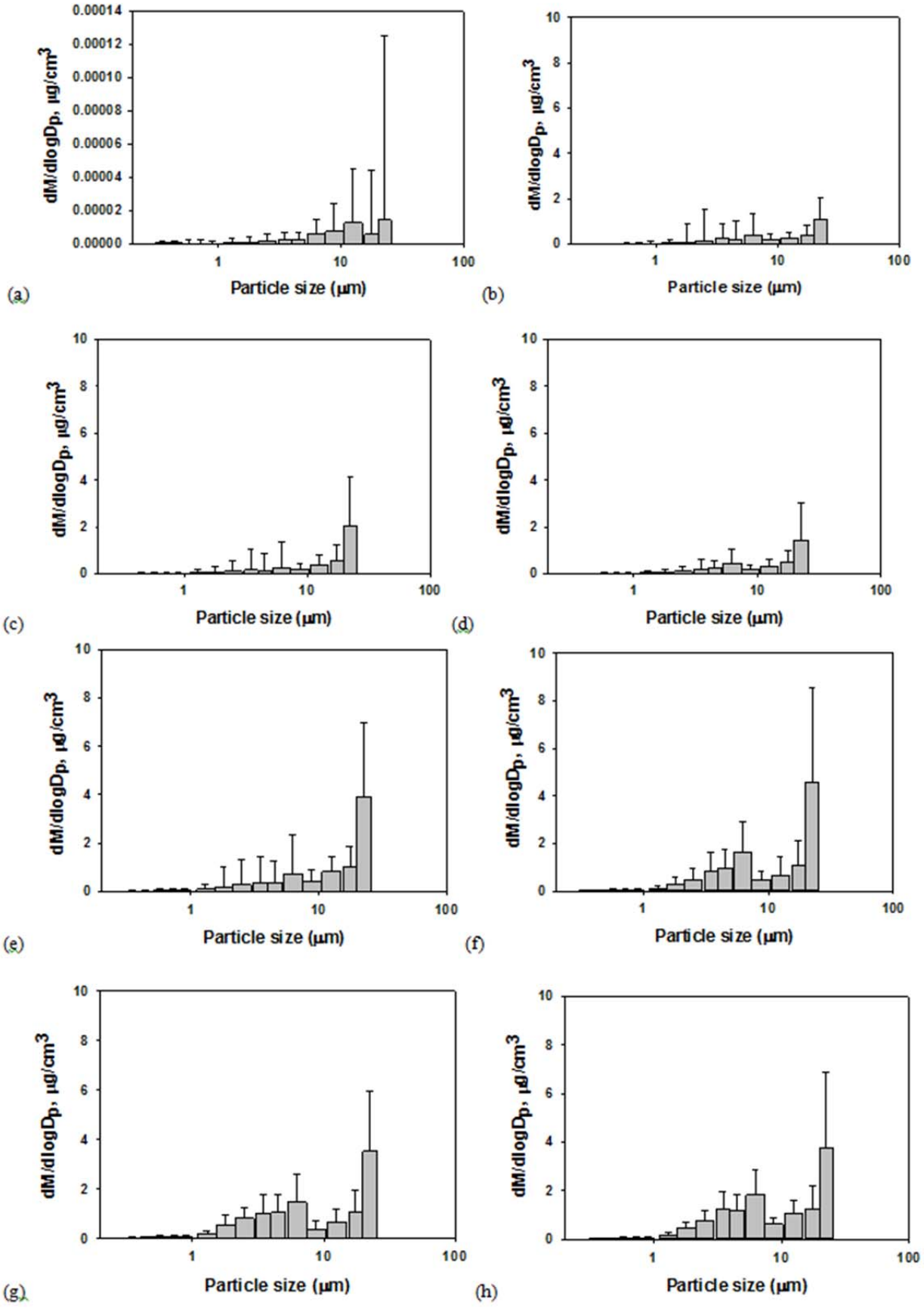
dM/dlogDp, µg/cm3
Particle size (µm)
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

FIGURE 2-7. Distribution of particle mass concentration ($dM/dlogD_p$) normalized by size measured (error bars represent 1 SD): (a), background air in chemical hood; (b), toluene at a flow rate of 0.193 ml $min.^{-1}$; (c), toluene at a flow rate of 0.380 ml $min.^{-1}$; (d), MDI working solution 1; (e), MDI working solution 2; (f), MDI working solution 3; (g), MDI working solution 4; (h), MDI working solution 5

Table 2-12. Summary of MDI particle mass distribution and statistics

Statistical parameter	Toluene 1	Toluene 2	MDI 1	MDI 2	MDI 3	MDI 4	MDI 5
MMD, μm	8.9	15.3	11.4	13.1	8.8	5.9	6.1
GSD	2.2	1.3	1.7	1.6	2.3	3.3	3.1
Total concentration, $\mu g\ cm^{-3}$	0.40	0.45	0.42	0.98	1.4	1.4	1.6

The MMD of toluene almost doubled in accordance with increasing the dispensed flow rate of the syringe pump from 0.193 to 0.380 ml $min.^{-1}$. This increase shows a direct dependence on the dispensed flow rate since the mass concentration did not change. Accordingly, polydisperse coagulation of these toluene aerosols may have occurred, especially since there is a broad range of particle sizes. The greater the difference in particle sizes, the greater the effect of coagulation. However, while polydisperse coagulation describes how particle size can change over time, the number concentration did not decrease as a function of increased mass concentration. Additionally, the wide distribution (GSD>2) was not reduced in relation to an increase in diameter of average mass. Theoretically, the distribution should have narrowed as a result of a competing mechanism characteristic of coagulation: a decrease in smaller particles and increase in the number of larger particles (Hinds 1999).The droplets produced from the nozzle of the IAGS have velocities much greater than their settling velocity, therby increasing the frequency of particle collisions (capture efficiency) (Hinds 1999). This type of coagulation is called kinematic coagulation. Frequency of collisions is increased based on flow velocity gradients. For example, droplets of various sizes will travel at different velocities along close

streamlines, producing a velocity gradient. Droplets on the faster streamline then collide with other droplets on a nearby slower streamline (Hinds 1999).

Some interesting effects of particle size and MDI concentration were observed in this study. As shown in Table 2-14, MMD augmentation was not observed in relation to increasing mass concentration of MDI in each successive working solution. Instead, the MMD calculated for working solutions 3-5 exhibited a decrease from the MMD related to working solutions 1 and 2. When compared to toluene dispensed at a flow rate of 0.193 ml $min.^{-1}$, the MMD of MDI working solution 1 (also delivered at a flow rate of 0.193 ml $min.^{-1}$) increased from 8.9 μm to 11.4 μm. This increase was consistent with the underlying rationale. Increasing the dispensed flow rate of MDI working solution 1 from 0.193 to 0.380 ml $min.^{-1}$ showed another increase in MMD; however, the magnitude of the increase was not consistent with that observed between toluene 1 and 2. This discrepancy may be a result of size resolution at the interval limits for the two bins differentiating between 12.5 and 15 μm.

Maintaining a dispensed flow rate of 0.380 ml $min.^{-1}$, and increasing the mass concentration should have promoted an increase in the observed MMD among working solutions 3-5 consistent with an increase in density and kinematic coagulation. However, a reduction, or shrinkage, of MMD is plausible with consideration of toluene evaporation. Increasing the MDI concentration may change the environment of the droplet (e.g., viscosity, solubility), which may have stabilized the formation of droplets. For example, Gupta et al. showed a reduction of particle size investigating efficacy of aerosolized prostaglandin E_1-loaded poly(lactic-*co*-glycolic acid) microspheres for treatment of pulmonary hypertension (Gupta, Rawat, and Ahsan 2010). Briefly, a water-in-oil emulsion was prepared with increasing concentrations of polyvinyl alcohol (PVA) in an external aqueous phase solution (via homogenization). Particle size

significantly decreased (e.g., from 10.7 to 2.4 μm) with higher PVA concentration in the water phase, which provided a hydrophilic environment that stabilized the emulsion droplets. Lee et al. attributed this effect to the larger size of the hydrophilic part of the PVA molecule than the hydrophobic part, which produced a particle curvature interface of smaller particles that favored higher surface PVA density (Lee et al. 1999). Consequently, the hydrophobic constituents were able to tightly pack at the core of the particle.

While droplets are not assumed to become more hydrophilic due to increasing concentrations of MDI (even though solubility of MDI and toluene are 0.2 and 0.07%), evaporation of toluene changes particle density, and may promote packing of the hydrophobic part of MDI. Since MDI and toluene were miscible, a network of similar intermolecular forces (e.g., van der Waals) was produced upon dissolution. Accordingly, toluene surrounded the aromatic components of MDI, as well as the NCO functional group. All five working solutions of MDI were considered undersaturated. Therefore, toluene was in excess of MDI; however, the volume of toluene decreased slightly with each successive working solution. Therefore, toluene was most likely at the surface of the droplet in accordance with solvation of MDI. Additionally, the surface of small droplets is sharply curved, modifying the attractive forces between surface molecules. Consequently, in the smaller droplets, especially with higher concentrations of MDI, toluene was likely evaporated off the droplet surface.

Assuming that the initial drop size leaving the nozzle is constant across all solutions, saturation ratio of evaporating toluene vapor was zero, and that presence of more MDI in the working solutions facilitated toluene evaporation, it is plausible that a response consistent with particle size reduction was observed. For example, the evaporation rate of a 10 μm particle was calculated at 10 seconds (equations 6 and 7).

(6)

$$T_{droplet}-T_{ambient}= \frac{(6.65+0.345T_{ambient}+0.0031Ta_{ambient}^{2})(S_r-1)}{1+(0.082+0.00782T_{ambient})S_r}$$

(7)

$$t= \frac{R\ p_p\ d_p^2}{8\ D_v\ M(p_d\ /\ T_d)}$$

where saturation ratio (S_r) was assumed 0 (no toluene present in the ambient air), and ambient temperature ($T_{ambient}$) was assumed room temperature (0°C, or 293K), gas constant (R) was 8.31 J/K, particle density (p_p) was 866.9 kg/m^3, particle diamter (d_p) was 10 μm, diffusion coefficient of toluene vapor at 293 Kelvin (K) was 8.7 x 10^{-6} m^2/s, molecular weight (M) was 0.09214 kg/mol, and droplet temperature (T_d) was determined to be 5.2 °C, or 278.2 K in equation 6.

The MMD may shrink according to the Kelvin effect with increasing mass concentration. Rader et al. determined the applicability between theory and measured evaporation rates of organic aerosol (Rader 1987). When the vapor pressure and diffusion coefficient were available, the agreement between theory and measured evaporation rates were good, which supports the Kelvin effect of organic aerosols. Therefore, if the droplet sizes were constant across all solutions then it would take less time to evaporate a 10 μm particle composed of both toluene and MDI than a pure toluene droplet. A shift in MMD towards smaller particle sizes would be the result of growing smaller MDI particles by increasing the concentration of MDI since it is not likely to evaporate at room temperature. Smaller particles of toluene may completely evaporate before entering distribution channels of the Grimm, thereby skewing the results towards a larger MMD. As shown in Figure 2-8, a trend of growing particles was observed less than 1 μm.

Since the atomizing nozzle was centered slightly inside the plane of the cassette during validation (i.e., to prevent internal wall losses of the aerosolized solution), isocyanate aerosol

samples were generated in direct line of the Grimm inlet to measure the size of wet particles leaving the IAGS nozzle; however, initial drop sizes were not measured. While not as close as the nozzle and GFF, the Grimm collected intermediate aerosol sizes (between initial drop and dry particle).

The MMD for a dry oil particle aerosolized from a working concentration of 11.0 μg/ml was 3.0 μm. Figure 2-9 provides a mass histogram of the data. The initial (wet) drop size of the 3.0 μm was calculated to be 127 μm from the volume fraction of the solution, volume of the dry particle, and volume of the wet particle. Due to a multitude of variables that must be considered when evaluating aerosol particle size, no MDI predictions were performed regarding dry particle size; however, these data shows that a significant drop in aerosol size occurs from the wet to dry state of the particle. This significant drop suggests that evaporation of toluene from the aerosol will leave behind a smaller MDI particle than the original aerosol mixture.

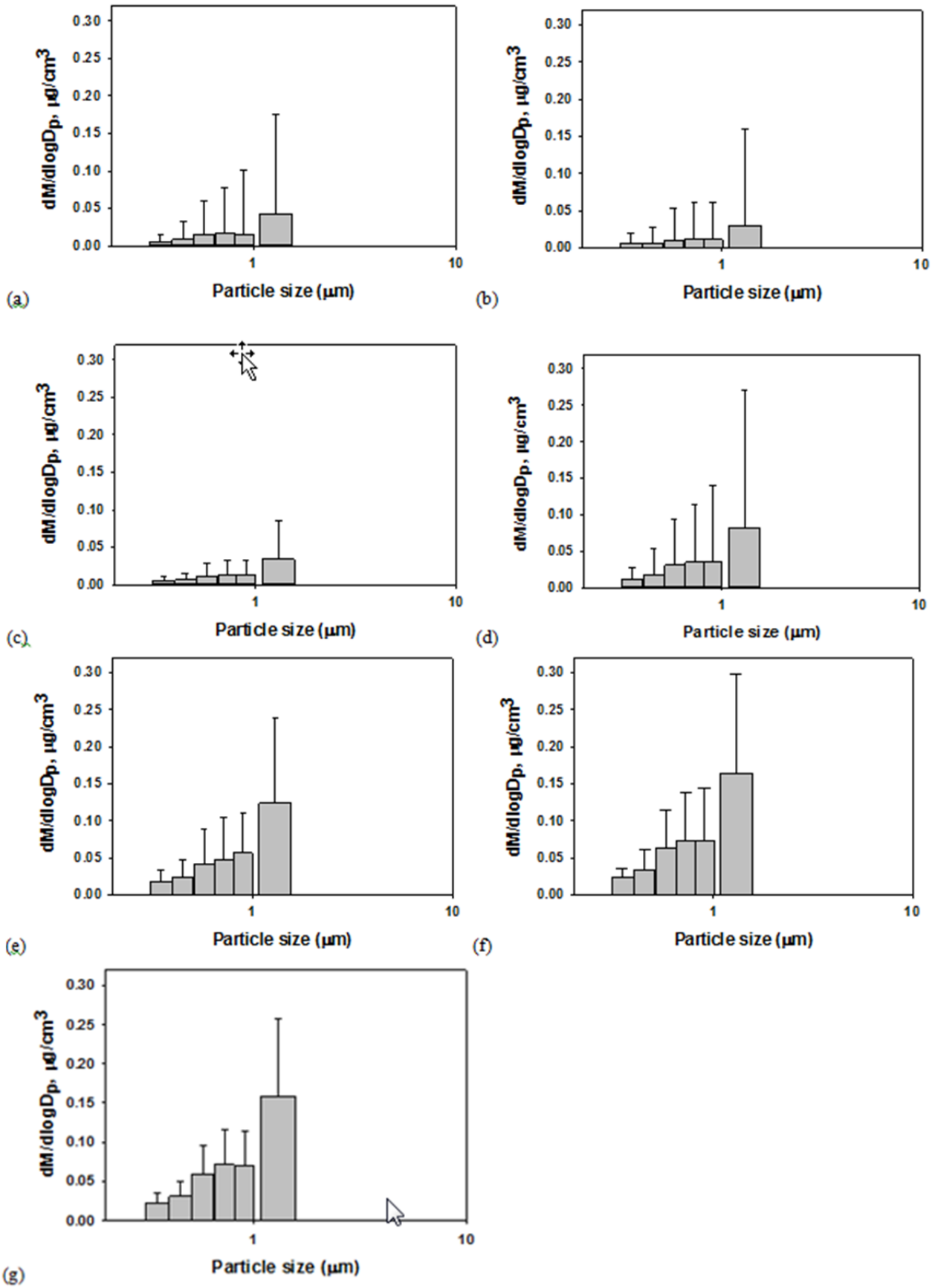
dM/dlogDp, µg/cm3
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1
10
Particle size (µm)
(a)
(b)
(c)
(d)
(e)
(f)
(g)

FIGURE 2-8. Distribution of particle mass concentration ($dM/dlogD_p$) normalized by size measured below 1µm (error bars represent 1 SD): (a), Toluene 1 (at a flow rate of 0.193 ml $min.^{-1}$); (b), Toluene 2 (at a flow rate of 0.380 ml $min.^{-1}$); (c), MDI working solution 1; (d), MDI working solution 2; (e), MDI working solution 3; (f), MDI working solution 4; (g) MDI working solution 5

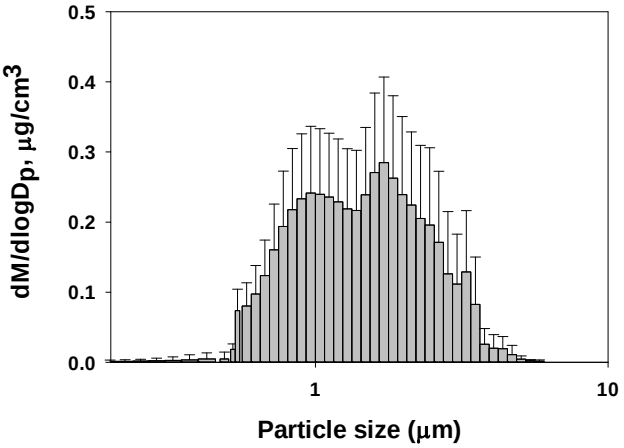

FIGURE 2-9. Distribution of particle mass concentration for oil/ethyl alcohol mixture ($dM/dlogD_p$) normalized by size measured

Since isocyanates were aerosolized in toluene and without the presence of a polyol, particle size determination using conventional gravimeteric methods (i.e., impactor) was challenging due to concerns of polymerization or curing of the isocyanate with moisture in the air. Lesage et al. determined a size distribution of polyurethane spray foam aerosols during application in residential construction using a Marple 8-stage impactor; however, net changes in filter weight due to isocyanate intermediate compounds (e.g., partial reacted products) were anticipated since this was a fast curing formulation. The use of the Grimm provides very useful knowledge for aims 3 and 4 of this study regarding the particle sizes generated from the IAGS; however, the Grimm was developed as a dustmonitor for indoor air quality rather than characterizing particle sizes of chemical aerosols. The reduction in MMD with increasing mass

concentration of MDI remains unknown and warrants further investigation in the future using several spectrometers simultaneously, allowing measurement of IAGS aerosols over a broader size range. Regardless of the shrinking MMD, this evaluation showed that approximately 95% of the aerosol mass concentration was associated with particles greater than 2 μm while 95% of the aerosol number concentration was associated with particles less 2 μm. The majority of the number concentration (75%) was contained between 0.35 and 0.725 μm. Particles greater than 3.5 μm contained 75% of the mass during the process. Since 2 μm is the recommended upper limit for using GFFs, ideally, the MMD would have been closer to 1μm to evaluate accuracy of sampling airborne MDI and TDI. However, the IAGS has provided a basis to model future experiments that spray load filters with known amounts of isocyanate.

CONCLUSION

Overall, we demonstrated in this project that an isocyanate aerosol generating system could be developed for the purpose of spray loading GFFs with known amounts of analyte to evaluate accuracy. Theoretical diisocyanate mass can be calculated from defined variables, such as the concentration of the working solution, flow rate of the syringe pump, and total sampling time. By comparing observed to theoretical, accuracy and consistency of each diisocyanate field sampling method can be demonstrated. Single-flow operation should be used to deliver isocyanate-only solutions to evaluate the methods at specific quantities or concentrations of isocyanate. The dual-flow operation, which included mixing of isocyanate with the polyol, introduced too much uncertainty in recovered amount of isocyanate since the kinetics of polymerization at these low concentrations, as well as completion rate of reaction were unknown. Future studies will not only compare FD and LD methods, but also evaluate accuracy

of the GFF method using small amounts of MDI, which is essential to preventing sensitization in workers, as well as protecting previously sensitized workers.

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CHAPTER 3—ATOMIZATION OF METHYLENE BISPHENYL ISOCYANATE IN SPRAY-ON TRUCK BED LINER COMPONENT A: A LABORATORY EVALUATION OF TWO SAMPLING METHODOLOGIES FOR MONITORING WORKER EXPOSURES USING A NOVEL SPRAY-ON DELIVERY SYSTEM

SUMMARY

The goal of this research project was to evaluate the accuracy of field and laboratory desorption methods under laboratory conditions. Using a liquid spike and an isocyanate aerosol generating sytstem, filters were treated with underivatized technical and pure grade methylene bisphenyl isocyanate over a range of concentrations. Working solutions with increasing mass concentrations of methylene bisphenyl isocyanate were prepared to evaluate the desorption methods at small amounts of methylene bisphenyl isocyanate. A total of 191 samples of methylene bisphenyl isocyanate (i.e., technical and pure grade) were collected. Quantitative determinations of methylene bisphenyl isocyanate from spray loading (n=105) and pipette loading (n=86) were reported in units of mass. A statistically significant three-way interaction was detected in the application of both technical and pure grade methylene bisphenyl isocyanate, specifcially between loading mechanism, desorption methods, and loading concentration (p-value <0.05). Generally, pipette and spray loading a field and laboratory desorbed filter with a solution of underivatized MDI (i.e., technical and pure grade) yielded a significantly low percent recovery. For a fixed loading, statistically significant differences were detected between field and laboratory desorption methods (at specific loading concentrations) when either grade of methylene bisphenyl isocyanatewas sprayed onto a filter, but not when it was liquid spiked. Consistent with other studies, field desorption filters consistently yielded a higher percent recovery of methylene bisphenyl isocyanatet than laboratory desorption filters. Underestimations of methylene bisphenyl isocyanate in both field and laboratory desorbed filters are likely attributed to reactions with water vapor or other hydroxyl radicals that may be present and

simultaneously collected onto the filter. Compared to the technical grade methylene bisphenyl isocyanate results, pure grade methylene bisphenyl isocyanate was even further underestimated. The presence of other compounds in the technical grade may have partially shielded (since technical grade was still underestimated) the methylene bisphenyl isocyanatefrom other reactants, facilitated dispersion of the methylene bisphenyl isocyanate, or enhanced the analytical results.

INTRODUCTION

Methylene bisphenyl diisocyanate (MDI) is an inherently reactive chemical used extensively to manufacture a wide range of end-use products encountered everyday (e.g., do-it-yourself polyurethane spray foam and paints) (Lesage et al. 2002; Boutin et al. 2005; Karol 1986; Allport 2003). Characterized by at least two isocyanate (NCO) functional groups, MDI is capable of step polymerization when mixed with a polyhydroxyl alcohol (polyol). Predominantly, MDI is used to produce polyurethane polymers; however, MDI may also be used to react with other materials containing hydroxyl groups (e.g., cellulose) to form non-polyurethane products (Booth et al. 2009; Allport 2003). Application of MDI to engineered wood products (e.g., particleboard, oriented strand board, medium density fiberboard) or sand-based foundry molds generate an adhesive; curing of MDI takes place in a continuous press operated at high temperature and pressure (Allport 2003; Booth et al. 2009).

Polyurethanes can be produced for specific applications and emerging technologies in construction, automotive, furniture and bedding, and mining (Lofgren et al. 2003; Allport 2003; Ashida 2007; Ulrich 1996; Raulf-Heimsoth and Baur 1998; Cummings and Booth 2002; Kang 2010; Kim 2008). Variations (e.g., functionality) of MDI and polyol formulations help manufacture polyurethanes with different specifications and properties, such as mechanical

strength, rigidity, or flexibility (Kang 2010; Kim 2008; Allport 2003; Ulrich 1996; Ashida 2007). Other chemicals (e.g., chain extenders, cross-linkers, and blowing agents) may be added to the reaction to reduce density while increasing stiffness of the polymer, as well as thermal and acoustic insulation (Allport 2003; Booth et al. 2009). According to the 2004 End-Use Market Survey conducted by the Center for the Polyurethanes Industry of the American Chemistry Council, MDI is used mainly to produce elastomers, reaction injection moldings, thermoplastic urethanes, and rigid foams (Council 2004).

The morphology of MDI-based rigid polyurethanes typically exhibits a closed cell structure with superior mechanical properties (e.g., high compression strength, high strength-to-weight ratio, and low moisture permeability) and thermal stability (e.g., low thermal conductivity) (Kim 2008; Kang 2010; Lofgren et al. 2003). Accordingly, these material properties are used in a multitude of consumer and industrial applications, which include protective coatings for walls, decks, boats, as well as insulation materials for construction and industrial applications, such as refrigerators, freezers, piping, tanks, and shipbuilding (Allport 2003; Lofgren et al. 2003; Boutin et al. 2005; Kim 2008; Kang 2010).

The term MDI is non-specific, which is used to describe all types of MDI-related species. During manufacturing of MDI, isomers and variants of the chemical are formed. The principal reaction product in MDI manufacturing is known as polymeric MDI (or technical grade MDI), which is an undistilled mixture containing approximately 50% oligoisocyanates and 50% pure MDI (Twitchett 1974; Allport 2003).

Oligoisocyanates are relatively low molecular weight homopolymers with a functionality of 3 or higher (Streicher et al. 2000; Ulrich 1996). These homopolymers are derived from condensation of 15 monomeric units (or less) of MDI (Streicher et al. 2000; Allport 2003; Bello

et al. 2004). Possessing multiple free iscoyanate functional groups, oligoisocyantes are also called polyisocyanates. MDI polyisocyanates are used to form polymeric products of great complexity by reacting with polyfunctional alcohols or amines (Bello et al. 2004).

Pure MDI usually refers to the monomeric form, 4,4' methylene bis-(phenyl isocyanate) (CAS Number 101-68-8), with a molecular weight of 250 amu (Booth et al. 2009; Allport 2003). However, a small percentage of isomers are also produced during manufacturing of pure MDI, which include 2, 4'- and 2, 2'-MDI. Pure MDI consists of at least 95% of the 4,4'-MDI isomer, which is achieved through distillation (e.g. continuous thin film or climbing film vacuum) (Streicher et al. 2000; Ulrich 1996).

Monomeric MDI is bi-functional, containing two isocyanate (NCO) functional groups attached to an aromatic parent compound in the para-position (Streicher et al. 2000; Deschamps et al. 1998; Nakashima 2002; Weyel and Schaffer 1985; Bello et al. 2007; Woolrich 1982; Bello et al. 2004). As a result of electrophilic aromatic substitution, MDI contains a net positive charge on each carbon atom of the functional group. Accordingly, polyols serve as nucleophilic agents, promoting addition of hydroxyl groups across the N=C bonds (Ulrich 1996). This reaction forms repeat units of NHCOO groups, which is a universal feature of all polyurethane products (Ashida).

The chemistry of polyurethane manufacturing covers a variety of processes and isocyanate handling, including how the isocyanate is supplied and reacted (Allport 2003; Booth et al. 2009). Depending on the end-use product, processes may be open or closed systems, and MDI may be heated or sprayed, or both. For example, continuous foaming lines of slabstock for making blocks of rigid or flexible foam are enclosed with either permanent or removable barriers (Cummings and Booth 2002).

Production of insulation or paints typically involves heating and spraying MDI as a two-component system in a continuous process. Two-stream metering allows long production runs of the same polyurethane product. Individual supply and metering of MDI and polyol blend permits uninterrupted production of different products with each change in formulation of either component.

During application of spray polyurethane foam insulation, a two-component system is used (Lesage et al. 2007). Before each component is mixed, a proportioning unit pumps MDI and a polyol mixture from separate containers in a desired mixing ratio. An independent heating system uniformly warms each component as it travels in a separate hose before entering a mixing head, or spray gun. Upon exiting an atomizing nozzle, the sprayed mixture begins reacting, and is cured within a few minutes.

Spray-on truck bed lining (STBL) is another industrial process involving a two-component spray application of isocyanate-containing materials. STBL has gained popularity in the past 6 to 7 years and are estimated to involve 10,000 workers in over 2000 franchises nationwide (Chester et al. 2005; OSHA Compliance Issues, Isocyanate Exposure in Autobody Repair and Collision Center 2006). Monomeric MDI is a main constituent in the isocyanate portion, which is heated and pressurized to specifications indicated by supplier.

MDI has an especially low vapor pressure (e.g., 0.000005 mm Hg at 25 °C) as compared to different isocyanates, but remains primarily an inhalation hazard due to mechanical aerosolization and heating during application. In 2003, Lofgren et al. assessed and characterized the MDI hazard for workers in the STBL industry using Washington State Occupational Safety and Health Administration (OSHA) inspection files and industrial insurance records (Lofgren et al. 2003). Data from 13 employers of small companies and specialty shops were examined. A

variety of products were used in these shops with reported MDI monomer concentrations as high as 75 percent in the spray component. Seven worker exposures were found above the OSHA ceiling limit of 200 $\mu g/m^3$ while five MDI-related insurance claims were filed at four inspected sites, two for new-onset asthma. Companies were cited for deficient respiratory programs and engineering controls for MDI.

STBL exposure to MDI presents serious worker health concerns as it may lead to either short- or long-term health effects such as asthma, airway irritation, hypersensitivity pneumonitis, and irritation of skin and mucous membranes. [14, 20] For some sensitized individuals, acute exposure may prove fatal (Chester et al. 2005). For instance, in 2003, a work-related fatality occurred from an acute asthmatic reaction following exposure from spraying an isocyanate-containing bedliner (Chester et al. 2005). Contact dermatitis and irritation to mucous membranes are less common, but may result in symptoms such as a rash, itching, hives, swelling of extremities, and irritation or serious burns to the eyes. [14, 20]

In general, diisocyanates are one of the most common causes of occupational asthma, but mechanisms of disease pathogenesis remain poorly understood (Wisnewski and Jones 2010). The reaction between MDI and a polyol is exothermic, consequently providing the heat necessary for volatilization (Streicher et al. 2000). Therefore, end-users that spray MDI-based systems may encounter aerosol exposures from mechanical atomization of the material, as well as vapor exposures during exothermic reactions. Based on the interplay of factors such as, route and duration of exposure, peak versus average exposure, chemical composition and physical form of isocyanate, and deposition site in the respiratory tract, partitioning value of isocyanate vapors, and host susceptibility—health mechanisms remain equivocally defined (Meredith, Bugler, and Clark 2000; Ott, Diller, and Jolly 2003; Bello et al. 2004).

The Occupational Safety and Health Administration (OSHA) mandates a ceiling permissible exposure limit (PEL) for airborne, monomeric MDI of 200 micrograms per cubic meter ($\mu g/m^3$), which is equivalent to 20 parts per billion (ppb) (Administration). However, this PEL is based on an antiquated American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value (TLV) list, which prescribes exposure criteria for only monomeric MDI using a ceiling limit of 20 ppb (Bello et al. 2004; Administration). OSHA defines a ceiling value as an exposure limit that shall not be exceeded at any time during the working day for a particular substance (Administration). OSHA does not mandate a TWA standard for MDI; however, the protocol for monitoring MDI is to assess the ceiling over a 15-minute period (Bello et al. 2004). Consequently, instantaneous concentrations may exceed the ceiling limit, while the average may very well be within the legal requirements of 20 ppb for airborne aromatic diisocyanates. This underestimation is an important caveat in regulatory compliance and sampling strategy for both MDI since peak exposures may be more relevant than lower long-term, or cumulative exposures in terms of risk for developing symptoms of asthma (Allport 2003; Mapp et al. 1988; Mapp et al. 2005; Chan-Yeung and Malo 1995).

Accurate measurements of MDI in workplace atmospheres are crucial, especially for previously sensitized workers. Quantitative determinations rely on capturing a representative sample; however, isocyanates characteristically have a widely varying disposition in the workplace, especially in spray applications (Pronk et al. 2006; Redlich and Karol 2002; Rom and Markowitz 2007; Streicher et al. 2000; Allport 2003; Bello et al. 2004). Technical difficulty in quantitative determinations of MDI are due to episodic exposure profiles, high reactivity and adsorptivity to particulate matter, particle size and concomitant exposure to other isocyanate

species, solvents, and other interfering additives (Allport 2003; Streicher et al. 2000; Redlich and Karol 2002; Lesage et al. 2007; Streicher 1994).

Currently, active sampling is typically achieved by use of an impinger filled with an absorbing solution or a reagent-coated filter sampler. These two sampling systems contain a derivatizing agent to enrich analytical identification and quantitation of isocyanates based on the formation of a stable urea derivative with strong molar absorptivities [5, 22].

Derivatizing agents are typically primary or secondary aliphatic amines. [5] Common derivatizing agents include tryptamine, 1-(2-methoxyphenyl) piperazine (MOPP), and 1-(9-anthracenylmethyl) piperazine (MAP) as listed in the National Institute of Occupational Safety and Health (NIOSH) Manual of Analytical Methods for isocyanates; 9-(methylaminomethyl) anthracene (MAMA), which is used in the SKC Inc. Iso-Chek method; and, 1-(2-pyridyl) piperzine (1-2PP) as used in OSHA Method 47 for MDI (Products ; SKC ; Administration ; Administration).

Hardy and Walker introduced 1,2-PP in 1979, which replaced earlier derivatizing agents, ethanol and nitroreagent (Hardy 1979). Analytical detection of the isocyanate functional group was enhanced with the development of 1,2-PP by improving sensitivity and selectivity of MDI. Putatively, 1,2-PP prevents competitive loss of the NCO group to polyols and/or water, and the resulting urea derivatives are detectable at low concentrations (Streicher et al. 1996). The physicochemical properties of 1-2PP afforded greater stability (e.g., 283°C bp), a wider range of miscibility in polar and non-polar solvents (including water), and less susceptibility to light degradation than the nitroreagent (Hardy 1979). With negligible steric hindrance, isocyanate groups react rapidly.

While a variety of derivatizing agents, and sampling and analytical methods exist for measuring airborne concentrations of isocyanates in the workplace, certain methodologies may be preferentially selected depending on the process. NIOSH recommends an impinger method, specifically NIOSH Method 5525, during work operations that spray fast-curing isocyanates, especially when sampling times are not adjusted to the product half-life (Streicher 1994; Streicher et al. 2000; Lesage et al. 2007). Fast-curing isocyanates may be lost to competitive reactions that can occur between collection and post-sampling procedures instead of reacting with the derivatizing agent. The impinger uses a solvent medium to trap, dissolve, and stabilize the isocyanate aerosols to help prevent an underestimation of isocyanates (Streicher et al. 2000). The use of excess reagent in the solution enhanced derivatization kinetics and quantitative recovery. Flow rate and specific dimensions of the impinger (e.g., jet diameter and strike distance) govern sampling efficiency, as well as impaction and diffusion mechanisms of particles and gases (Allport 2003).

Impinger methods are also recommended for processes that generate particles greater than 2μm in diameter (Streicher 1994; Streicher et al. 2000). Impingers prevent the passage and allow dissolution of isocyanate particles greater than 2μm in diameter. A study by the International Isocyanate Institute (III) compared the collection efficiencies of GFFs and impingers using inert polyethylene glycol aerosols inside a wind tunnel (Hext et al. 2003). GFFs collected higher amounts of particles with an aerodynamic diameter ranging from 0.1 to 3 μm while 60-65% of the particles passed through the impinger. The impinger demonstrated a collection efficiency greater than 100% when sampling larger particles with a diameter between 5 and 20 μm. Therefore, depending on the application of the isocyanate, sampling results may not accurately represent true concentration of either MDI and over exposures may go unnoticed.

Although impinger methods are preferred, their use is often inconvenient and may prove unsuitable for certain conditions. For instance, volatile solvents used in the impinger evaporate and must be refilled. Additionally, impingers are inherently hazardous as workers are potentially exposed to solvents such as toluene and dimethyl sulfoxide. Furthermore, impinger solvents are potentially flammable.

OSHA modified the original impinger method by substituting a 37-mm cassette containing a glass fiber filter (GFF) impregnated with 1-2PP for the impinger device. Since OSHA has the authority to enforce occupational exposure limits, isocyanate personal samples are usually collected using this system. OSHA recommends drawing a known volume of air through an open-face cassette at 1 liter per minute (L/min) for a total volume of 15 liters (L). The OSHA Sampling Method 47 for MDI uses 1.0 milligram (mg) of the secondary amine to coat the GFF (Administration ; Administration). Following the completion of sampling, the OSHA recommends replacing the top cover and small plugs on the cassette, and sending to an accredited analytical laboratory. Upon receipt, the laboratory will remove the GFF from the cassette, placing it into a vial containing 2 ml of 90/10 ACN/DMSO desorbing solution.

Anecdotal evidence gathered in GFF evaluations of 1-2PP while sampling airborne isocyanates revealed several limitations. One group determined that the reagent readily sublimes from the GFF during long sample times, especially in hot, humid weather (Allport 2003). As much as 67% of the 1.0 mg of reagent coated on the filter evaporated off after a four-hour sample time that was initiated to comply with the 1989 PEL, which increased the sampling air volume from 15 L to 240 L. Another study demonstrated capillary transfer of 1-2PP as high as 85% in 24 hours from the GFF to a mixed cellulose ester (MCE) backup pad. Recommended

corrective actions included suspending the filter in the middle of the cassette with no back-up pad, or replacing the MCE with a stainless steel backup pad.

The OSHA validated sampling and analytical method 47 using GFFs coated with 1,2-PP and an HPLC with a fluorescence detector (Administration). Experimental designs were tailored to evaluate retention and extraction efficiency, detection limits, reliable quantitation limit, thermostability, and storability. Controlled atmospheres of MDI were not generated. Instead a liquid spiking technique was used as an alternative to study the behavior of MDI once collected in an open-face cassette.

Working standards of MDI-urea derivatives were prepared to avoid polymerization of the isocyanate during evaluations. Briefly, re-crystallized MDI and 1,2-PP were mixed to form white slurry. Purified MDI derivative was obtained following precipitation, filtration, and hexane washing of the slurry. Stock solutions were prepared using DMSO. Subsequent dilutions of the stock were made using ACN to arrive at the working range of MDI. A conversion factor of 0.4339 was calculated by dividing the molecular weight of MDI by the molecular weight of the MDI-urea derivative. The amount of free MDI was then determined by multiplying the weight of MDI derivative by this conversion factor.

Retention efficiency was determined by liquid spiking GFFs with a target amount of MDI using the conversion factor. For example, 9.9μg of MDI derivative was delivered to six GFFs, which was equivalent to 4.3μg of MDI per filter. Subsequently, 20 L of air at 80% relative humidity and 22°C was drawn through the filters to determine retention of the analyte. The average percent recovery of MDI from the filters was 97% using an HPLC and fluorescence detector.

Extraction efficiency for method 47 was determined in a similar approach as above. A target concentration of MDI was delivered to 14 filters; however, no air was drawn through the filter. The extraction efficiency was 96.3%.

The retention and extraction efficiencies for OSHA Sampling Method 47 were determined under conditions that facilitated high recoveries of MDI, which do not accurately reflect reaction variables present in the field. For example, preparation of MDI derivatives in a solution environment provided optimal derivatization kinetics, especially without the presence of competitive reactions. Additionally, the solution provided mobility of the MDI-urea standards to evenly distribute across the filter. Previous studies have demonstrated that reagent-coated filters were generally found to yield higher amounts of isocyanates than impingers during side-by-side collection of laboratory-generated MDI atmospheres (Seymour 1987; Coyne 1993; Tucker 1982; Huynh 1992). During field comparisons (e.g., during application of MDI-based polyurethane roof, pouring of MDI at factories and foundries, spray painting) of these samplers, impingers were more likely to give higher results of isocyanates due to the presence of of competitive reactions (Wu 1991; Health. 1984; Andersson 1983; Seymour 1987; Rosenberg 1984). Streicher et al. reasoned that, under laboratory conditions, higher results were attained by filter methods because derivatization kinetics were unchallenged, and therefore unimportant (Streicher, Kennedy, and Lorberau 1994).

Lesage et al. evaluated the efficacy of impinger and filter samplers in a side-by-side comparison during application of polyurethane spray foam (a two-component spray system) inside five single-family homes under construction (Lesage et al. 2007). Personal sampling of applicator exposures (n=13) reported a range of airborne MDI concentrations from 0.07 to 2.05 mg/m^3. Almost two-thirds of the particle mass concentration of the spray foam aerosol was

greater than 3.5 microns in diameter, and approximately 20% of the fractions collected were respirable. Results from filter sampling methods indicated a 6% to 40% underestimation of airborne MDI concentrations compared to concentrations determined by the impinger. The authors attributed this difference to the observed particle size distribution.

Isocyanate accessibility to the derivatizing agent is critical when using reagent-coated GFFs, especially in two-component spray applications that consist of an isocyanate and polyol mixture (Booth et al. 2009; Streicher 1994). These products rapidly cure with a half-life of less than two minutes. Micrographs of GFFs containing samples taken during spray applications show minimal contact with the reagent-coated fibers (Bell 1994). As a result, dispersal of the aerosol is negligible, and larger aerosols exhibit an inherent challenge of accessing the reagent (Streicher 1994). Consequently, isocyanates will be lost to competitive reactions within an aerosol mixture and underestimated.

In addition to aerosol particle size and fast cure times, airborne dust or particulate matter may physically hinder isocyanate groups from reacting with the derivatizing agent on the filter media (Karoly 1998; Booth et al. 2009). For instance, MDI may be used as a binder in the manufacturing of engineered wood (e.g., medium density fiberboard). Dust generated in wood mills during this application can impact the collection media and prevent contact of the isocyanate with the derivatizing agent. The isocyanate can be adsorbed onto the surface of the dust or particulate, and undergo a curing reaction before a urea derivative is formed (Booth et al. 2009).

An underestimation of isoycanate exposure puts the worker at a potential risk for a recurring overexposure. In order to preserve the MDI for quantitative analysis, the MDI and polyol need to be separated, or the curing reaction interrupted (Streicher 1994). Additionally,

reagent accessibility needs to be enhanced during fast cure times, generation of large aerosols (>2μm), or both (Streicher 1994).

To improve the performance of filter methods, the filter may be removed from the cassette in the field and desorbed immediately after sampling in a vial containing a solvent miscible with the reactants (Karoly 1998; Streicher et al. 2000). When the filter is desorbed, the extracting solvent will dissolve both the derivatizing reagent and any un-reacted isocyanate, allowing the two to combine in solution and form a stable urea-derivative. Streicher et al. recommend desorbing samples in the field immediately after sampling whenever isocyanates are collected (Streicher et al. 2000). However, OSHA still prescribes filter desorption to occur at the analytical laboratory upon receipt of sample shipment.

Existing literature suggests that a significant difference exists between the results of field- and laboratory-desorbed methods for airborne methylene bisphenyl diisocyanate. In an MDI stability study, Karoly collected side-by-side samples at four different wood mills using a company-specific sampling and analytical method (ICI Polyurethanes sampling and analytical Method 1024G, revision 1.7) (Karoly 1998). Briefly, samples were collected on 13-mm GFFs containing the derivatizing agent MOPP and 2% diethyl phthalate from one to three hours. Sample preparation included one field desorbed (FD) and one laboratory desorbed (LD) filter within each sample set. FD filters were removed from the cassette, rinsed, and immersed in a vial containing derivatizing agent and toluene while LD samples were sealed in the cassette. Both sets of samples were sent to an analytical laboratory.

A statistically significant difference was observed using the Wilcoxon signed-rank test to compare FD and LD analytical results (Karoly 1998). Higher amounts of MDI were yielded from desorption of filters in the field as compared to desorption at a laboratory. These results suggest

that dusty environments encountered at wood mills interfere with derivatization of the isocyanate on a GFF. Accordingly, higher amounts of MDI were achieved through the FD technique, which promoted efficient mixing between MDI-coated dust particulates and MOPP.

Recently, Schaeffer et al. conducted a side-by-side comparison of personal breathing zone samples collected from applicators in nine STBL businesses in northern Colorado. The aim of this study was to determine if a statistically significant difference existed between the OSHA Sampling Method 47 and the Wisconsin Occupational Health Laboratory (WOHL) sampling method LC 48, which recommends field desorption. These two methods are commonly used by consultation and compliance. Briefly, the OSHA sampling method 47, and the WOHL sampling method LC 48 are identical in their "upstream" sampling procedures for monomeric MDI, but deviate in post-sampling preparation prior to analysis.

A wide range of exposure concentrations was observed during application of the two-part urethane coating. Additionally, 15% of all samples collected (n=72) exceeded the OSHA ceiling PEL while compliance for three sample sets was a function of method selection. For example, an overexposure was determined from an FD result while the LD result suggested a possible overexposure.

Schaeffer et al. determined a significant difference between the OSHA and WOHL sampling methods for airborne MDI in STBL work atmospheres. The field-desorbed sampling methodology yielded consistently higher MDI concentrations than the laboratory-desorbed sampling methodology, which suggests that immediate desorption minimizes isocyanate loss and potential underestimations. Spray droplets and large particles have been conjectured as a limitation of the reagent coated GFFs due to minimal contact between the isocyanate and the coated fibers. Since STBL application generates aerosols with a wide range of particle sizes

containing a mixture of MDI and polyol, these results were not unexpected. Additionally, results from the analysis of variance also indicated that the effect of company was significant, meaning that different facility factors and environmental conditions within each company, such as the use of ventilation or humidity level, affected the MDI concentrations; indicating the potential for better mitigation of exposures using the hierarchy of controls.

Since this study was conducted in the field, the true concentration of MDI present during sampling was unknown. Without having a true concentration against which to compare sample results, true accuracy of each method could not be determined, only that a difference between the two methods existed.

While variants of sampling methods for MDI exist for particular workplace environments (Streicher et al. 2000), companies have greater incentive to use the OSHA methods in order to comply with current regulation. However, in addition to a solution environment that provided optimal derivatization kinetics during validation of GFF methods, aerosols of various particle sizes were not included in the evaluation of measurement accuracy. Since aerosols are a key constituent of a typical exposure scenario involving spray applications of isocyanates, especially MDI, more research is needed to narrow the gap in understanding the effects of particle size on quantitative determinations of MDI.

The purpose of this research is to determine the accuracy of field desorption (FD) and laboratory desorption (LD) techniques using aerosolized MDI (underivatized) instead of a liquid spike of urea-bound MDI. Particle size will also be examined since these GFF methods are commonly used in two-component, polyurethane spray-applied systems even though impingers are the preferred method. To accomplish this goal, an isocyanate aerosol generating system (IAGS) was designed and tested to provide a more realistic evaluation consistent with typical

isocyanate exposure scenarios. This is the first attempt, to the author's knowledge, to evaluate these methods using atomized MDI instead of a liquid spike.

Briefly, pure and technical grade MDI will be delivered to a series of GFFs. Accuracy will be evaluated on the basis of how closely quantitated results from FD and LD sampling techniques replicate theoretical results. Using both the IAGS and a liquid spike technique, we anticipate that FD results will closely approximate theoretical amounts of MDI while LD results will significantly underestimate MDI due to inherent challenges to stabilize the chemical, allowing MDI to cure from competitive reactions rather than derivatize. Furthermore, with the absence of other compounds present in technical grade MDI, pure grade MDI is conjectured to react faster with the derivatizing agent, 1,2-PP, yielding higher amounts of MDI.

Consistent with other investigations, our hypothesis is in that isocyanate aerosol size will impact collection and extraction efficiency, and FD techniques, while not the preferred method, is an amenable approach to monitor applications that generate fast-reacting isocyanate aerosols. Resolution of accuracy between these two methods will provide a basis for better exposure mitigation through the use of effective control measures capable of reducing airborne MDI to a protective level.

MATERIALS AND METHODS

Chemicals and Equipment

Pure MDI (4,4'-methylenbis(phenylisocyanate) was purchased from Sigma-Aldrich (St. Louis, MO). Technical grade MDI was obtained from a local Line-X Company (Loveland, Colorado) along with the material safety data sheet. BDH® American Chemistry Society (ACS) grade toluene (CAS No. 108-88-3), suitable for histology and cytology application, was acquired

from the Colorado State University Environmental Health Services' chemical redistribution program (unopened) (Fort Collins, CO).

A KDS 200 Two-Syringe Infusion Pump was purchased from KD Scientific (New Hope, Pa.). An EZ-STARTER airbrush set with an atomizing nozzle was purchased from PAASCHE® Airbrush Company (Chicago, IL), which included air hose with couplings that connected to a ¼ inch Victor® CGA 346 two-stage gas regulator (Denton, Texas) fitting assembled to a size 300 grade D high pressure breathing air purchased from Airgas (Fort Collins, Colorado).

The Wisconsin Occupational Health Laboratory (WOHL) provided 37 mm, 3-piece cassettes containing glass fiber filters (GFF) treated with one milligram (mg) of 1, 2- pyridyl piperazine along with desorption vials containing two milliliters (ml) of 90% acetonitrile and 10% dimethyl sulfoxide (90/10 ACN/DMSO).

Description of Isocyanate Delivery System Design

A small-scale spray system (Figure 3-1) was designed and built to load GFFs with MDI test aerosols to evaluate percent recovery (see Chapter 2). Briefly, the isocyanate aerosol generating system (IAGS) was validated at a dispensed flow rate of 0.193 and 0.380 ml $min.^{-1}$ using water. An experimental value of less than 1% error was determined at items A-C (Figure 3-1) of the IAGS. A subsequent pilot study, consisting of five samples, was conducted using an MDI working solution corresponding to 1.3 μg ml^{-1} of toluene dispensed at a flow rate of 0.193 ml $min.^{-1}$. This pilot study was a proof of principle and not an evaluation of the GFF method. With a standard deviation of 18 nanograms (ng) and a coefficient of variation of 8%, the IAGS was concluded to exceed expectations of consistent delivery of aerosolized MDI in order to evaluate the GFF method.

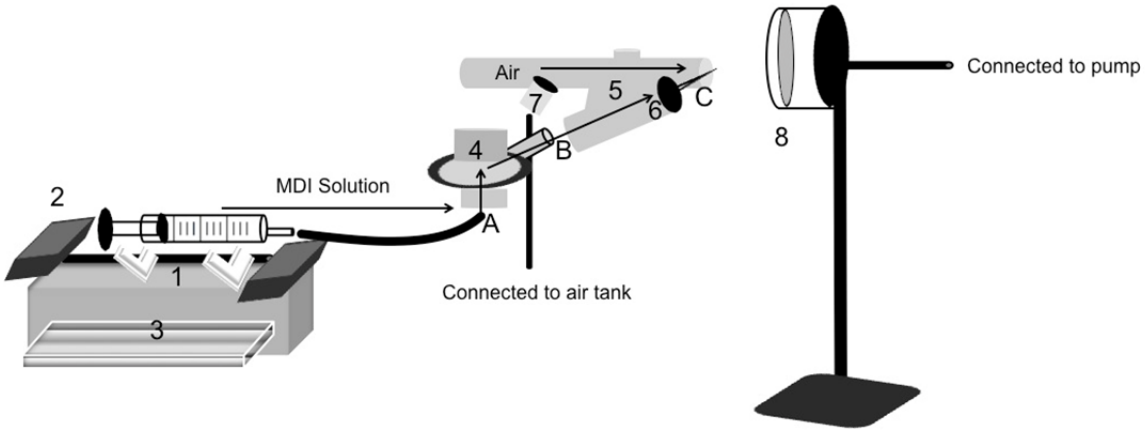

FIGURE 3-1. MDI Delivery System and Sample Collection: 1, the barrel of the syringe rests in the "V" and a retaining arm (not shown) secures the syringe in place; 2, pusher block mechanism that travels along guide rods when drive nut is engaged and depresses syringe plunger at set rate; 3, keypad used to navigate displayed menu features and selection of functions (e.g., syringe manufacturer, size, and diameter, units and rate settings, and directional mode); 4, airbrush adaptor; 5, airbrush; 6, adjustable nozzle; 7, compressed air adaptor; 8, 37-mm open-face cassette; A, connection to the airbrush adaptor; B, connection of adaptor to the airbrush; C, exit out of the nozzle.

Toluene was determined not to interfere with the reaction between MDI and 1, 2-PP based on the reported amounts of MDI following a series of dilutions with an increasing volume of toluene (see Chapter 2). Aerosolized samples were collected using the GFF FD sampling methodology and sent to the Wisconsin Occupational Health Laboratory (WOHL) for HPLC quantitation. A trend of decreasing MDI with respect to the dilution factor was detected in each set of MDI samples (i.e., pure and technical grade).

Using a Grimm Dust Monitor Model 1.108 (Grimm Technologies, Inc., Douglasville, GA), the IAGS particle size distribution was determined. Number concentrations reported by the Grimm were converted to a mass concentration using the following equations:

(1)

$$\text{Density}_{\text{mixture}} = 1/(\%_{\text{mdi}} / \text{density}_{\text{mdi}}) + (\%_{\text{toluene}} / \text{density}_{\text{toluene}})$$

(2)

$$dM_i = \pi/6\ (\rho_p D^3_{p,i})\ dN_i$$ (Peters, Ott, and O'Shaughnessy 2006)

where ρ_p represented particle density, and $D^3_{p,i}$ and dN_i represent the midpoint aerodynamic diameter and number concentration measured in the *i*-th channel of the Grimm (Peters, Ott, and O'Shaughnessy 2006).

Equation 1 was used to calculate the density of each MDI working solution, and equation 2 was used to calculate the particle mass concentrations at each size distribution channel of the Grimm (see Chapter 2). Approximately 95% of the aerosol mass concentration was associated with particles greater than 2 μm while 95% of the aerosol number concentration was associated with particles less 2 μm. The majority of the number concentration (75%) was contained between 0.35 and 0.725 μm. Particles greater than 3.5 μm contained 75% of the mass during the process

The fundamental assumptions of this research were:

- The IAGS will deliver atomized MDI consistent with the reported accuracy and reproducibility of the KD Scientific 200 Two-Syringe Infusion Pump
- Toluene is miscible with MDI making a homogenous solution of working standards
- Toluene does not interact with the derivatizating agent, 1,2-PP
- A wide range of MDI particle sizes will remain on the filter
- By comparing observed amounts of MDI to theoretical values determined from the syringe pump, concentration of working solution, and sample time, accuracy and consistency of each diisocyanate field sampling method can be demonstrated.

Preparation of MDI Solutions

Technical grade MDI:

A local Line-X Company (Loveland, Colorado) provided commercial MDI, PCS 454 A (Eteco, Inc), along with a corresponding material safety data sheet (MSDS). The w/w% of monomeric MDI (CAS No. 101-68-8) reported in the MSDS was less than 50%. Other chemicals reported were a proprietary component (25-45% w/w), and modified MDI (less than 10% w/w). A sample of this commercial MDI was shipped to the WOHL for an independent analysis of the composition. The WOHL determined that PCS 454 A actually contained 0.53 grams of MDI per gram (g) of material using high-performance liquid chromatography (HPLC) methods, which was slightly higher than described by the MSDS. Therefore, in order to prepare 10mL of a 1mg/mL MDI stock solution, approximately 18.9 mg of PCS 454 A was weighed out on a Denver Instrument M-series 220D analytical balance and mixed with 10mL of toluene (equation 3). This solvent is readily miscible with isocyanates, and conjectured not to interfere with derivatization of the isocyanate.

(3)

1mg/mL x 10mL = 10mg of MDI;
10mg of MDI from component A requires a
total of 18.9mg based on 1/0.53 x 10mg

From the 1mg/ml MDI stock solution, working toluene solutions containing monomeric MDI concentrations corresponding to 1.3, 2.6, 5.2, and 10.4 micrograms (μg) ml^{-1} (Table 3-1) were made using equation 4 (and confirmed by HPLC at the WOHL):

(4) $Concentration_1 \times Volume_1 = Concentration_2 \times Volume_2$

TABLE 3-1. Technical grade MDI solutions prepared using equation 2.

Category	MDI concentration
Working solution 1	1.3 μg ml^{-1}
Working solution 2	2.6 μg ml^{-1}
Working solution 3	5.2 μg ml^{-1}
Working solution 4	10.4 μg ml^{-1}

This log-scale of MDI concentrations was initially based on a dispense flow rate of 1 ml $minute^{-1}$. Accordingly, an IAGS runtime of 1min. would deliver microgram amounts of MDI comparable to the amount the OSHA used in examining retention efficiency. Using a Mines Safety Appliances (MSA) Electronic Laminar Flow (ELF) pump to collect 20 L of air, concentrations of airborne MDI near the OSHA ceiling PEL could be achieved. However, solutions were prepared and confirmed by HPLC at the WOHL before validation of the IAGS was completed.

Since the IAGS was validated at 0.193 and 0.380 ml $min.^{-1}$, working solution 1 was metered at both dispense flow rates to maintain an approximate logarithmic scale. Subsequently, workings solutions 3-5 were metered at 0.380 ml $min.^{-1}$. As a consequence, amounts of MDI delivered were approximately 20% and 40% of the original amount based on these adjusted flow rates.

Application of these MDI working solutions required increasing the sampling rate of the MSA ELF pump while decreasing air volume to achieve practical concentrations of airborne MDI. Briefly, the recommended air volume and sampling rate in OSHA method 47 was 15 L and 1 L/min. To approach the OSHA ceiling PEL, only 2 L of air were collected at 2 L/min. For example, if working solution 1 was delivered at a flow rate of 0.193 mL $minute^{-1}$ for 1 min., theoretically 250.1 ng (equation 5) was delivered to a GFF, which is the equivalent to 15 ppb (equation 6).

(5) MDI solution ($\mu g\ ml^{-1}$) x dispensed flow rate ($ml\ min^{-1}$) x sample time (minute) = μg

(6) $mg\ m^{3\ -1}$ = ppm (MW)/24.45 (P_o/P_m) (T_m/T_o)

	$mg\ m^{-3}$ =	Mass concentration based on HPLC results and volume of air collected
	ppm=	parts per million
	MW=	Molecular weight of MDI
where :	P_o=	Normal pressure
	P_m=	Measured pressure
	T_o=	Normal temperature
	T_m=	Measured temperature

Pure MDI:

To prepare pure MDI standards, a 1mg/ml stock solution was prepared. Briefly, 38.4mg were weighed and dissolved in 39 ml of toluene. From the 1mg/ml MDI stock solution, working toluene solutions containing monomeric MDI concentrations corresponding to 1.4, 2.7, 5.4, and 11.0 $\mu g\ ml^{-1}$ (Table 3-2) were made using equation 4 (and confirmed by HPLC at the WOHL).

TABLE 3-2. Pure grade MDI solutions prepared using equation 2

Category	MDI concentration
Concentration #5	1.4 $\mu g\ ml^{-1}$
Concentration #6	2.7 $\mu g\ ml^{-1}$
Concentration #7	5.3 $\mu g\ ml^{-1}$
Concentration #8	11.0 $\mu g\ ml^{-1}$

Study Design

Accuracy of FD and LD methods was evaluated using underivatized monomeric MDI in the form of an aerosol -, and liquid-spiking technique. Methods were assessed on their capability to collect, derivatize, and quantify ultra-trace amounts (i.e., ng) of MDI. A logarithmic scale of MDI concentrations was used to determine if accuracy was modified or influenced by an interaction with concentration. A comparison between spray and pipette loading was included based on environmental differences between each application. Briefly, spray loading of MDI on

a filter will produce discreet droplets in the immediate vicinity of limited 1,2-PP. Conversely, spiking a solution of analyte provides mobility to both MDI and reagent, which evenly distributes these reactants across the working area of the filter. Thus, a solution environment was created, which optimized derivatization kinetics between 1,2-PP and MDI. Percent recovery results from liquid spiking underivatized MDI were compared to the OSHA results from pipette loading filters with pre-derivatized MDI.

Collection of Atomized Monomeric MDI

Technical and Pure Grade

Atomized MDI was collected from five different concentrations using an open-face sampling technique described in OSHA Method 47; however, flow rate of the sampling pump connected to the cassette was operated at 2 liters per minute (L/min) instead of 1 L/min. With this adjusted flow rate, practical airborne concentrations in parts per billion were achieved within a short sample time of one minute. A Mine Safety Appliances (MSA) Escort Electronic Laminar Flow (ELF) pump was calibrated at a flow rate of 2 L/min using a Dry Cal DC Lite-DCLT 5K rev 1.08 (Pompton Plains). One sample was collected at a time, and the flow rate was checked post-sampling to ensure they were within ±5% of the original flow rate. The MDI sampling cassettes and desorbing solution were stored in a refrigerator before use as prescribed by OSHA method 47 and WOHL method LC48.

Although a net increase in filter weight as a result from aerosolized water was not included in the validation of the IAGS, filters were still weighed using a Denver Instrument M-series 220D analytical balance (Arvada, CO) before and after collection of aerosolized isocyanates. For example, after a 1 min. sample time, a filter was immediately disconnected from the pump and weighed. Initially, the mass reading on the balance stabilized for approximately

five seconds and this output was recorded. Subsequently, the mass reading on the balance started to wane after stabilization, indicating evaporation. Logically, evaporation was occurring the entire time of sample collection; however, weighing the filter was still a useful metric to ensure comparable aerosol mass concentrations were delivered to each FD and LD filter. Briefly, since each sample was handled the same, the rate of evaporation was assumed equal during and after application. Therefore, while variance in replicates was expected due to evaporation, filter weights should be within two standard deviation of the mean for comparability. Additionally, knowing the net increase in filter weight at each flow rate provided a standard that was used to accept or reject samples based on reproducibility. Outliers were rejected on the basis of not being within in two standard deviations, most likely due to the presence of an air bubble in the system. An air bubble may be introduced when loading syringes, which would cause a break in continued flow of isocyanate. Measures were taken to minimize the presence of air bubbles. In the event an air bubble was present, the system was voided until the air bubble was cleared.

After the sampling cassettes were removed from the apparatus and weighed, the top cover and small plugs of the LD cassette were replaced. The FD filter was removed using forceps and placed into a glass vial containing 2 mL of 90/10 ACN/DMSO desorbing solution. The researcher agitated the vial gently to ensure that the entire filter was saturated with desorbing solution. The vial was sealed with the cap provided by WOHL and wrapped with parafilm around the top of the vial to prevent leaking.

Accuracy of the FD and LD techniques was evaluated based on comparing HPLC-quantitated results from the WOHL, an American Industrial Hygiene Association (AIHA) accredited laboratory, to theoretical results. Based on the IAGS dispensed flow rate of an MDI concentration and sample time, a theoretical result was calculated (Table 3-3 and 3-4). By

comparing observed to theoretical, we can determine accuracy and consistency of each diisocyanate field sampling method.

TABLE 3-3. Theoretical amounts of technical grade MDI delivered by the IAGS based on concentration, flow rate, and time

Category	Concentration (μg/ml)	IAGS Flow Rate (ml/min)	Sample Time (min.)	Theoretical Amount of MDI (ng)
Working solution 1	1.3	0.193	1	231
Working solution 2	1.3	0.380	1	454
Working solution 3	2.6	0.380	1	1254
Working solution 4	5.2	0.380	1	2470
Working solution 5	10.4	0.380	1	4560

TABLE 3-4. Theoretical amounts of pure grade MDI delivered by the IAGS based on concentration, flow rate, and time

Category	Concentration (μg/ml)	IAGS Flow Rate (ml/min)	Sample Time (min.)	Theoretical Amount of MDI (ng)
Working solution 6	1.4	0.193	1	270.2
Working solution 7	1.4	0.193	1	532
Working solution 8	2.7	0.380	1	1026
Working solution 9	5.3	0.380	1	2014
Working solution 10	11.0	0.380	1	4180

Spray loading FD and LD filters using technical grade MDI were collected on two different days. All FD samples were collected in May 2010 while LD samples were collected in June, 2010. FD and LD pure grade MDI samples were collected on the same day within the month of November 2012. In one day, working solutions 6-8 were applied to FD and LD samples while concentrations 9 and 10 were collected the following day.

Liquid Spike Sample Collection of Monomeric MDI

Technical and Pure Grade:

Monomeric MDI was liquid spiked directly across a filter approximately at equal distances using a Mettler Volumate Pipettor, which was independently calibrated. Appendix A is a table illustrating pipette accuracy in a quality assurance exercise using water. Open-face cassettes were not connected to a sampling pump. While retention efficiency (percent of analyte remaining on the filter) was a concern over an allotted sampling time, the objective of this experiment was to attain a baseline of MDI quantitations under optimal kinetics (i.e., without humidity interference). FD and LD percent recovery results were compared to the OSHA's results from liquid spiking pre-derivatized MDI in their evaluation of retention efficiency. Technical and pure grades of MDI were included in this evaluation of FD and LD methods. After loading and weighing the cassette, FD and LD samples were prepared as described above.

Theoretical amounts of MDI were calculated based on MDI concentration and pipette volume. To be consistent with the IAGS flow rate, pipette volumes of 0.193 and 0.380 ml were used. Pipette loading of FD and LD filters using technical grade MDI occurred on the same day in the month of August 2010; however, since three months passed between spray and pipette loading, solvent evaporation was a concern. Accordingly, an aliquot of each concentration was sent to the WOHL for HPLC analysis. Technical grade standards of MDI became more concentrated due to solvent evaporation than previous standards prepared for spray loading (Table 3-5). Pure grade MDI standards were the same as those reported in Table 3-4.

TABLE 3-5. Theoretical amounts of technical grade MDI based on concentration (after evaporation) and pipette volume

Category	Concentration (μg/ml)	Pipette volume (ml)	Theoretical Amount of MDI (ng)
Working solution 1	1.2	0.193	290
Working solution 2	1.2	0.193	570
Working solution 3	3.3	0.380	1216
Working solution 4	6.5	0.380	2508
Working solution 5	12	0.380	4940

Indoor Air Quality

A QTrak Indoor Air Quality Monitor Model 8554 (TSI, Shoreview, MN, USA) was used to measure temperature and humidity. The QTrak was equipped with a thermistor and thin-film capacitive sensors to measure temperature and humidity, respectively. The thermistor sensor was capable of monitoring a temperature range of 0 to 50°C with an accuracy of ± 0.6 °C. The thin-film capacitive sensor for humidity was capable of monitoring 5 to 95% relative humidity (RH) with an accuracy of ± 3% RH. Using the data log mode, measurements were taken every 5 min. during sampling during application of pure grade MDI working solution only.

Statistical Analysis

Statistical analyses were performed using the Statistical Analysis System (SAS) computer program (version 9.2, SAS Institute Inc., Cary, NC). A sample size calculation and power analysis were not performed *a priori*. Briefly, variance and effect size of loading GFFs with underivatized MDI in a controlled setting were unknown. Therefore, only post-hoc analyses were conducted. To assess accuracy, p-values were used to compare differences between working solutions, desorption methods, grades of MDI, and loading mechanisms (i.e., spraying and pipetting). Values of $p < 0.05$ were considered significant.

Accuracy of desorption method was defined as 100% recovery of MDI compared to theoretical amounts; calculated from concentration and dispensed flow rate of the syringe pump. Briefly, comparing experimental results to theoretical produced a percent recovery for each FD and LD replicate. A scatterplot of studentized residuals versus predicted values was performed to assess normality and independence. In this plot, two outliers were identified in the spray loading data (Figure 3-2) associated with filter desorption in the laboratory. Specifically, one filter was detected in working solution 3 and another in working solution 4. The amount of MDI reported from these filters was approximately two standard deviations less than the mean. These outliers invariably corresponded to a nominal net increase in filter mass compared to all other replicates (Tables 3-17 and 3-18). For example, the outlier in working solution 3 (filter 2) had a net increase of 17 mg. The other four replicates demonstrated an increase of approximately 90 mg. With a standard deviation of 31 mg, the net increase in filter mass of filter 2 was greater than two standard deviations from the mean. Accordingly, an air bubble within the system most likely occurred, causing a gap in continuous flow of MDI. Since there was no reason for such variance in a physical experiment, the two outliers were removed from post-hoc analyses.

Following removal of two outliers, log-transformed data showed the residuals to be approximately normal and independent. Accordingly, a log-based ratio of zero represented 100% recovery based on taking the logarithm of 1, denoting an experimental amount equal to the theoretical value. Using the log-based ratio as the dependent variable in the statistical model, FD and LD differences from zero were analyzed using least square means to determine if a statistically significant difference existed at each concentration for a fixed loading.

General Linear Models were used to assess the multivariable relationship between loading mechanism, desorption method, and concentration. Least square means of the log-based

ratio and standard error were used to compare the mass (i.e., ng) of MDI collected by FD and LD methods at each loading concentration. To investigate interactions between each variable (i.e., loading mechanism, desorption method, and loading concentration), a three-way analysis of variance (ANOVA) was performed with four degrees of freedom. Power was increased using a three-way parametric ANOVA since the loading mechanism and desorption method were categorical variables.

To determine differences between pure and technical grade MDI, a common model four-way ANOVA was utilized using SAS version 9.3. Results from these two grades of MDI were evaluated to demonstrate if composition of MDI interacted with loading mechanism, desorption method, or loading concentration, or in some iteration of these variables.

RESULTS AND DISCUSSION

A total of 191 MDI (i.e., technical and pure grade) samples were collected using FD and LD methods. Quantitative determinations of MDI from spray loading (n=105; Table 3-6) and pipette loading (n=86; Table 3-7) were reported in units of mass (ng/sample) and concentration ($\mu g/m^3$) based on the air volume pulled through the cassette. Descriptive statistics of FD and LD MDI data collected at each concentration are presented as the mean ± standard deviation and range (Tables 3-8-3-11).

TABLE 3-6. Summary of spray loading FD and LD samples

Grade of MDI	Loading mechanism	Desorption method	Working solution	Total
Technical	Spray	Field	1	5
Technical	Spray	Field	2	5
Technical	Spray	Field	3	5
Technical	Spray	Field	4	5
Technical	Spray	Field	5	5
Technical	Spray	Laboratory	1	5
Technical	Spray	Laboratory	2	5
Technical	Spray	Laboratory	3	4*
Technical	Spray	Laboratory	4	4*
Technical	Spray	Laboratory	5	5
Pure	Spray	Field	6	5
Pure	Spray	Field	7	6**
Pure	Spray	Field	8	5
Pure	Spray	Field	9	5
Pure	Spray	Field	10	5
Pure	Spray	Laboratory	6	5
Pure	Spray	Laboratory	7	6**
Pure	Spray	Laboratory	8	5
Pure	Spray	Laboratory	9	10**
Pure	Spray	Laboratory	10	5

* Outlier removed

** Additional samples collected to evaluate toluene evaporation from filter

TABLE 3-7. Summary of pipette loading FD and LD samples

Grade of MDI	Loading mechanism	Desorption method	Working solution	Total
Technical	Pipette	Field	1	4
Technical	Pipette	Field	2	4
Technical	Pipette	Field	3	3
Technical	Pipette	Field	4	3
Technical	Pipette	Field	5	4
Technical	Pipette	Laboratory	1	4
Technical	Pipette	Laboratory	2	4
Technical	Pipette	Laboratory	3	3
Technical	Pipette	Laboratory	4	3
Technical	Pipette	Laboratory	5	4
Pure	Pipette	Field	6	5
Pure	Pipette	Field	7	5
Pure	Pipette	Field	8	5
Pure	Pipette	Field	9	5
Pure	Pipette	Field	10	5
Pure	Pipette	Laboratory	6	5
Pure	Pipette	Laboratory	7	5
Pure	Pipette	Laboratory	8	5
Pure	Pipette	Laboratory	9	5
Pure	Pipette	Laboratory	10	5

TABLE 3-8. Summary of analytical results from spray loading test filters without outliers

Grade of MDI	Working Solution	Desorption Method	Mean Mass (ng) (± SD; range)	Theoretical Mass (ng)	Ratio (Observed/ Theoretical)
Technical	1	Field	318 (±36; 280-360)	231	1.37
Technical	2	Field	454 (±29; 410-490)	456	0.995
Technical	3	Field	1100 (±71; 1000-1200)	1254	0.079
Technical	4	Field	2140 (±55; 2100-2200)	2470	0.866
Technical	5	Field	3940 (±55; 3900-4000)	4560	0.865
Technical	1	Laboratory	248 (±8; 240-260)	231	1.07
Technical	2	Laboratory	436 (±30; 410-480)	456	0.956
Technical	3	Laboratory	1010 (±62; 960-1100)	1254	0.805
Technical	4	Laboratory	1900 (±82; 1800-2000)	2470	0.769
Technical	5	Laboratory	3780 (±164; 3600-3900)	4560	0.828
Pure	6	Field	260 (±16; 240-280)	270.2	0.962
Pure	7	Field	390 (±50; 310-460)	532	0.733
Pure	8	Field	808 (±99; 650-920)	1026	0.787
Pure	9	Field	1700 (±71; 1700-1800)	2014	0.844
Pure	10	Field	2960 (±230; 2600-3200)	4180	0.708
Pure	6	Laboratory	212 (±14; 190-230)	270.2	0.784
Pure	7	Laboratory	398 (±158; 340-430)	532	0.748
Pure	8	Laboratory	776 (±300; 730-810)	1026	0.756
Pure	9	Laboratory	1640 (±712; 1500-1700)	2014	0.814
Pure	10	Laboratory	2740 (±89; 2600-2800)	4180	0.655

TABLE 3-9. Summary of LD analytical results of spray loading technical grade MDI working solutions 3 and 4 with outlier

Working solution	Desorption Method	Mean Mass (ng) (± SD; range)	Theoretical Mass (ng)
3	Laboratory	920 (±208; 560-1100)	231
4	Laboratory	454 (±29; 410-490)	456

TABLE 3-10. Summary of airborne concentrations from spray loading test filters

Grade of MDI	Loading Mechanism	Desorption Method	Airborne Concentration (ppb) (± SD; range)
Technical	Spray	Field	15.6 (±1.8; 14-18)
Technical	Spray	Field	22.2 (±1.5 20-24)
Technical	Spray	Field	52.8 (±2.9; 50-56)
Technical	Spray	Field	104 (±5.5; 100-110)
Technical	Spray	Field	194 (±5.5; 190-200)
Technical	Spray	Laboratory	12.2 (±0.45; 12-13)
Technical	Spray	Laboratory	21.4 (±1.7; 20-24)
Technical	Spray	Laboratory	44.6 (±10; 27-52)
Technical	Spray	Laboratory	87.6 (±12.9; 65-9)
Technical	Spray	Laboratory	186 (±5.5; 180-190)
Pure	Spray	Field	12.8 (±0.84; 12-14)
Pure	Spray	Field	18.7 (±2.3; 15-22)
Pure	Spray	Field	39.6 (±4.8; 32-45)
Pure	Spray	Field	82 (±3.6; 77-86)
Pure	Spray	Field	146 (±11.4; 130-160)
Pure	Spray	Laboratory	10.3 (±0.73; 9.3-11)
Pure	Spray	Laboratory	19.5 (±1.4; 17-21)
Pure	Spray	Laboratory	37.6 (±1.7; 35-39)
Pure	Spray	Laboratory	79 (±3.7; 74-82)
Pure	Spray	Laboratory	138 (±8.4; 130-150)

TABLE 3-11. Summary of analytical results from pipette loading test filters

Grade of MDI	Working Solution	Desorption Method	Mean Mass (ng) (± SD; range)	Theoretical Mass (ng)	Ratio (Observed/Theoretical)
Technical	1	Field	265 (±6; 260-270)	290	0.913
Technical	2	Field	527 (±15; 510-540)	570	0.924
Technical	3	Field	1066 (±57; 1000-1100)	1216	0.876
Technical	4	Field	2266 (±152; 2100-2400)	2508	0.903
Technical	5	Field	4450 (±129; 3900-4000)	4940	0.901
Technical	1	Laboratory	277 (±5; 270-280)	290	0.955
Technical	2	Laboratory	530 (±11; 520-540)	570	0.929
Technical	3	Laboratory	1100 (±0; 1100-1100)	1216	0.904
Technical	4	Laboratory	2300 (±0; 2300-2300)	2508	0.917
Technical	5	Laboratory	4575 (±50; 4500-4600)	4940	0.926
Pure	6	Field	260 (±16; 240-280)	270.2	0.895
Pure	7	Field	390 (±50; 310-460)	532	0.763
Pure	8	Field	808 (±99; 650-920)	1026	0.754
Pure	9	Field	1700 (±71; 1700-1800)	2014	0.774
Pure	10	Field	2960 (±230; 2600-3200)	4180	0.631
Pure	6	Laboratory	212 (±14; 190-230)	270.2	0.903
Pure	7	Laboratory	398 (±158; 340-430)	532	0.774
Pure	8	Laboratory	776 (±300; 730-810)	1026	0.746
Pure	9	Laboratory	1640 (±712; 1500-1700)	2014	0.784
Pure	10	Laboratory	2740 (±89; 2600-2800)	4180	0.612

Percent Recovery of Technical Grade MDI

In this analysis, the mean FD and LD data were compared to theoretical amounts of technical grade MDI to obtain a percent recovery, or a ratio of observed values to theoretical values. A ratio of one represented a 100% recovery of MDI. A percent recovery, least squares mean (LSMEAN), and attendant p-value is shown for each loading mechanism, desorption method, and working solution of MDI in Tables 3-12 and 3-13.

TABLE 3-12. LSMEANs associated with pipette loading technical grade MDI

Loading	Desorption	Working Solution	Log 10_ratio LSMEAN	Pr> |t|	Percent recovery
Pipette	Field	1	-0.03922944	0.0005	91.4%
Pipette	Field	2	-0.03378640	0.0026	92.5%
Pipette	Field	3	-0.05733845	<0.0001	87.7%
Pipette	Field	4	-0.04460807	0.0007	90.4%
Pipette	Field	5	-0.04550408	<0.0001	90.1%
Pipette	Laboratory	1	-0.01918853	0.0797	95.7%
Pipette	Laboratory	2	-0.03167630	0.0046	93.0%
Pipette	Laboratory	3	-0.04354089	0.0009	90.5%
Pipette	Laboratory	4	-0.03759970	0.0036	91.7%
Pipette	Laboratory	5	-0.03335545	0.0029	92.6%

TABLE 3-13. LSMEANs associated with spray loading technical grade MDI

Loading	Desorption	Working solution	Log 10_ratio LSMEAN	Pr> |t|	Percent recovery
Spray	Field	1	0.13664567	<0.0001	138%
Spray	Field	2	-0.00071423	0.9411	99.5%
Spray	Field	3	-0.05762568	<0.0001	87.7%
Spray	Field	4	-0.06239630	<0.0001	86.6%
Spray	Field	5	-0.06350208	<0.0001	86.4%
Spray	Laboratory	1	0.03064319	0.0023	107%
Spray	Laboratory	2	-0.01835787	0.0613	96%
Spray	Laboratory	3	-0.09457504	<0.0001	80.5%
Spray	Laboratory	4	-0.11424453	<0.0001	76.9%
Spray	Laboratory	5	-0.08180508	<0.0001	82.9%

Spray and Pipette Loading Technical Grade MDI

The least squares means (LSMEAN) were calculated (Tables 3-12 and 3-13) using the general linear model (GLM) to integrate the effects of desorption method, loading mechanism, and loading concentration.

A total of 86 observations were collected, but only 84 observations were analyzed in the model after removing two outliers at approximately -4 and -6 observed on the studentized residual versus predicted plot (Figure 3-2). After removing these outliers, distribution of data was concluded to be approximately lognormal.

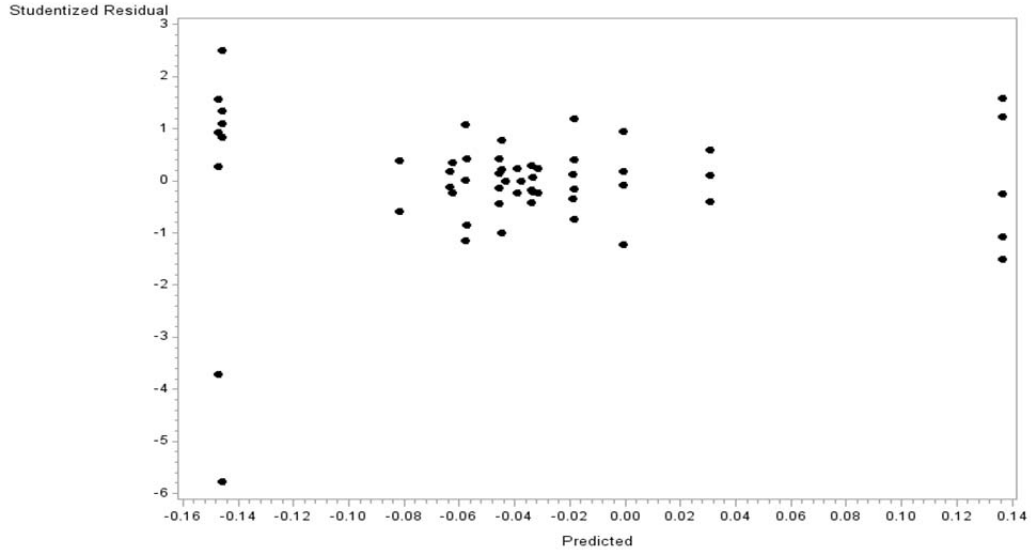

FIGURE 3-2. Studentized residual versus predicted plot of technical grade MDI data with two outliers.

Using the general linear model, relationships between analytical results and individual variables were analyzed. Since data were unbalanced in this model (i.e., fewer pipette replicates than spray loading), type I and III sums of squares were calculated to determine the presence of interactions. Significant effects (p-value<0.05) of desorption and concentration was demonstrated in the test of main effects. With a p-value of 0.089, the effects of loading were not

considered significant. While greater than the alpha level of 0.05, a p-value of 0.089 suggests practical relevance and should be considered to play a role in the outcome of MDI results.

The GLM demonstrated the presence of significant interactions ($p<0.05$), including two- and three-way interactions between variables (Table 3-14). Therefore, interpretations of these interactions supplant any further analysis of the main effects. All three variables were analyzed for statistical interaction to see if the amount of MDI was the same at different levels of each variable (i.e., two-way interaction). In this analysis, the variable of loading MDI by either spraying or pipetting modified analytical results in each method of desorption and across all loading concentrations.

TABLE 3-14. Iterations of two-way and three-way interactions and results.

Interaction	Degrees of Freedom	Type I Sums of Squares (p-value)	Type III Sums of Squares (p-value)
Loading*Desorption	1	0.01443570 (<0.0001)	0.01656294 (<0.0001)
Loading *Concentration	4	0.07242828 (<0.0001)	0.07237240 (<0.0001)
Desorption*Concentration	4	0.00588720 (0.0194)	0.0044330 (0.0597)
Loading*Desorption*Concentration	4	0.00765876 (0.0049)	0.00765876 (0.0049)

A statistically significant three-way interaction was detected between loading mechanism, desorption methods, and loading concentration (p-value <0.05) (Table 3-15). Two-way interactions were analyzed for variance across levels of a third variable. This three-way interaction was not unexpected. Analytical results at each concentration were conjectured to vary with desorption method and loading mechanism (Figures 3-3 and 3-4). These results are consistent with previous work conducted in field experiments. For example, filters desorbed in the field versus the laboratory produced statistically significant higher amounts of MDI in the

two different investigations. Additionally, pipette loading of free MDI onto a filter was anticipated to play a pivotal role in quantitative determinations, achieving higher recoveries of MDI than spray loading.

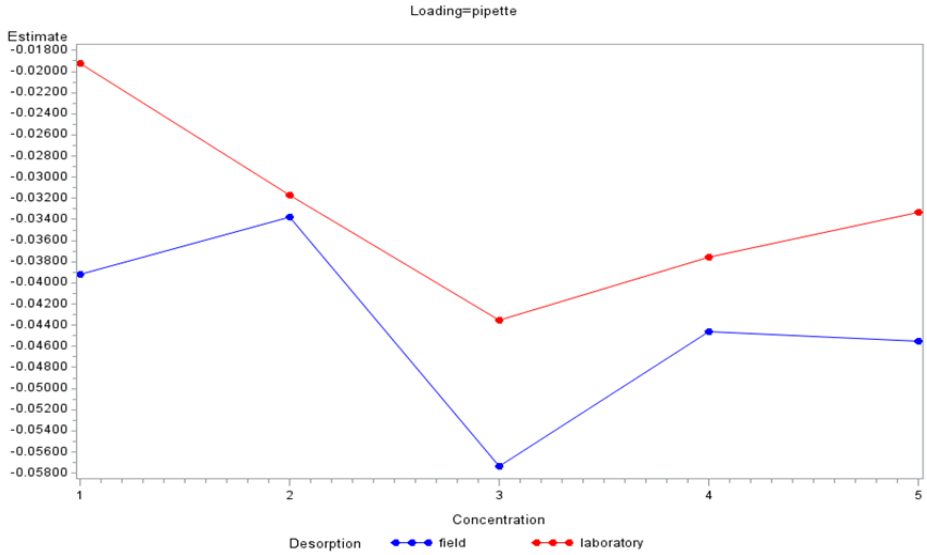

FIGURE 3-3. Three-way interaction of pipette loading technical grade MDI

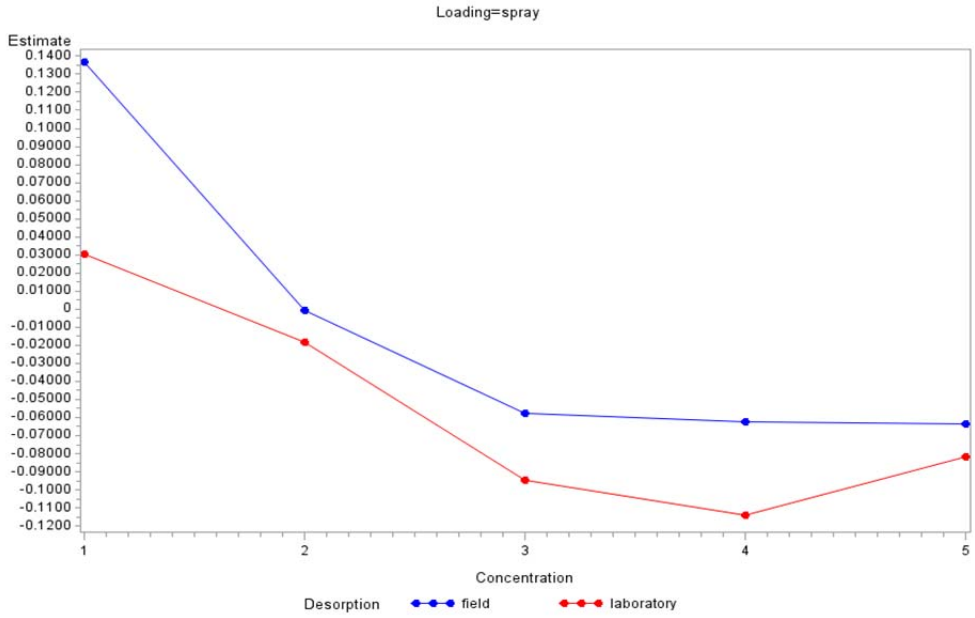

FIGURE 3-4. Three-way interaction of spray loading technical grade MDI

This is the first study to atomize underivatized MDI in a controlled setting to investigate percent recovered as compared to spiking a solution of underivatized MDI. As stated earlier, a three-way interaction was detected between the variables. Therefore, analytical results varied with the effects of loading concentration of MDI, desorption method, and loading mechanism (aerosol versus liquid). Pipette loading filters was expected to yield higher amounts of MDI than spray loading. Briefly, collection of discrete droplets limited the access of MDI to the derivatizing agent while spiking a solution of MDI promoted a solution environment. Consequently, pipette loading allowed unreacted MDI to spread across the working area of the filter, increasing access to 1,2-PP. A solution environment provided optimal conditions for derivatization by increasing mobility of both 1,2-PP and isocyanate.

In the OSHA experiment, derivatized MDI was prepared and pipette loaded onto a GFF to eliminate competitive polymerization reactions. While this study demonstrated retention and extraction efficiency of an LD GFF with approximately 97% recovery, these results may not accurately reflect true recoveries due to the inherent reactivity of MDI. Since stable urea derivatives of MDI were loaded, losses or consumption to other reactions other than with 1,2-PP were not assessed in these experiments.

LSMEANs from pipette loading of underivatized MDI demonstrated a statistically significant difference from zero in both filter desorption methods except in LD LSMEAN in concentration 1 (Table 3-12, and Figure 3-5). Retrospectively, the LSMEANs correlate with a difference of observed MDI to theoretical, which ranges from 12ng to approximately 500ng in the raw data (Table 3-15).

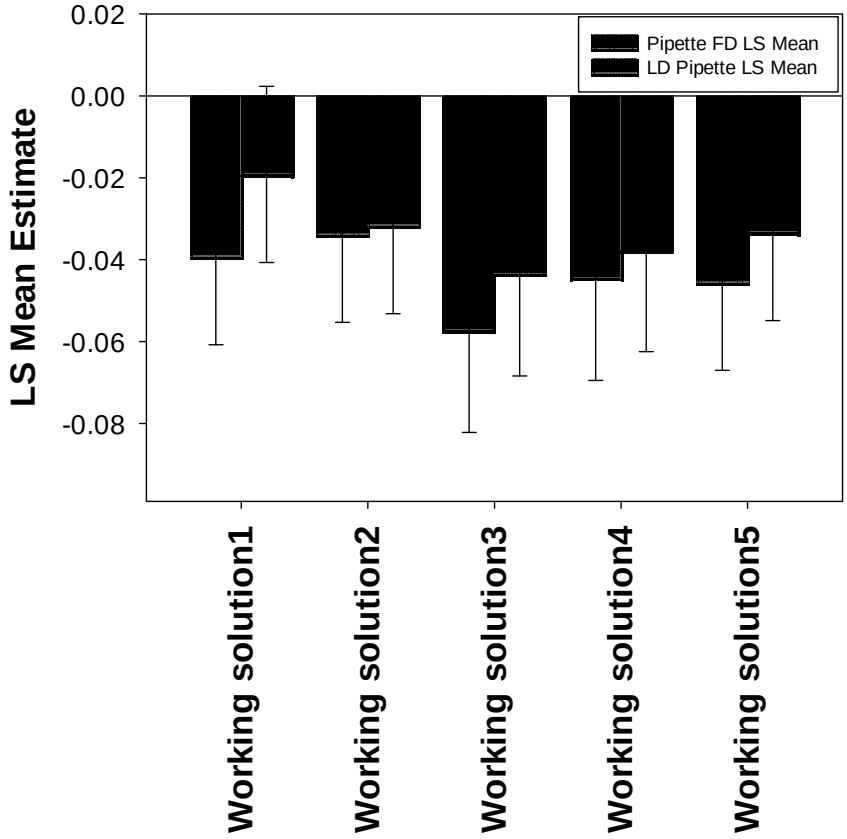

FIGURE 3-5. Comparison of FD and LD LSMEANS from pipette loading filters with technical grade MDI.

The mass difference between observed and theoretical amounts of MDI for each working solution and desorption method is shown in Table 3-15. The smallest mass difference from the theoretical amount was observed in the analytical results of LD filters treated with working solution 1. In contrast, the largest mass difference was demonstrated in FD filters treated with working solution 3. The LD and FD LSMEANs in working solutions 1 and 3 were -0.019 and -0.057 (Table 3-12). Only the FD LSMEAN from working solution 3 corresponded to a statistically significant difference from zero (Table 3-12). The average LD mass from working solution 1 was 278ng while the average mass from FD filters was 265ng. The mass difference between these two methods was approximately 13ng with a p-value of 0.193, which was a percent recovery difference of approximately 4%.

TABLE 3-15. Mass differences between observed to theoretical amounts of MDI pipette loaded with technical grade MDI.

Working solution	Theoretical amount of MDI	Mean FD difference from theoretical	Percent Difference	Mean LD difference from theoretical	Percent Difference
1	290 ng	-25 ng	9%	-12.5 ng	4.6%
2	570 ng	-42.5 ng	7.8%	-40 ng	7.3%
3	1216 ng	-149 ng	13%	-116 ng	10%
4	2508 ng	-241 ng	10%	-208 ng	8.6%
5	4940 ng	-490 ng	10%	-365 ng	7.7%

As with the liquid solution of monomeric MDI, collection of discreet droplets of MDI using the IAGS significantly underestimated expected amounts of MDI. An underestimation of MDI from LD methods was not unexpected; however, FD methods were conjectured to closely reflect theoretical amounts of MDI. FD and LD LSMEANs associated with loading concentration 2 did not show a statistically significant difference from zero. The FD LSMEAN was -0.0007 (Table 3-13), indicating a mass difference of only 2ng (Table 3-16) from expected amount of MDI; however, a difference of 20ng was detected in the LD recovery of MDI compared to the theoretical (Table 3-16). Poor recoveries of MDI were observed in LD filters loaded with concentrations 3 and 4, which corresponded to a difference of 334 and 690ng, and a percent difference of 22 and 26% (Table 3-16). These differences represented the greatest difference from zero as represented by the LSMEANs shown in Table 3-13.

TABLE 3-16. Mass differences between observed to theoretical amounts of MDI spray loaded with technical grade MDI.

Concentration	Theoretical	Mean FD difference from theoretical	Percent Difference	Mean LD difference from theoretical	Percent Difference
1	231	+87ng	32%	+17ng	7.1%
2	456	-2 ng	0.43%	-20ng	4.4%
3	1254	-154ng	170%	-334ng	22%
4	2470	-334ng	14%	-690ng	26%
5	4560	-620ng	14%	-780ng	19%

Table 3-13 also presents a curious phenomenon observed in concentration 1. All LSMEANs were negative, indicating an underestimation of MDI based on interplay of all variables except for spray results from loading concentration 1. These LSMEANs were positive, suggesting a percent recovery greater than 100% in both FD and LD methods. It is unclear why a larger amount than expected of MDI was observed at this loading concentration. Indoor air quality was not monitored during these experiments and all FD and LD blanks were collected post-sampling. Although care was taken to replicate experimental conditions among separate sample collections, study design precluded collection of FD and LD blanks during experimental setup. Residual airborne MDI from experimental setup may have been present during collection of loading concentration 1. However, since loading concentrations were sprayed from least (1.2 μg/ml) to most concentrated (10.4 μg/ml), it is unlikely that such a low concentration would have persisted.

Samples of MDI working solutions were shipped to the WOHL for HPLC quantitation. These samples were aliquoted directly from the primary container containing each solution. Since quantitation occurred in tandem with GFF samples, the expected or theoretical amount of MDI was accurate; however, syringes were loaded with working solutions from a separate container (e.g., beaker) than directly from the stock to avoid contamination. It is possible that

during initial set up, evaporation of toluene from working solution 1 occurred from this secondary container and not from the primary container stored in a -20 °C refrigerator. Evaporation of toluene would have increased the amount of analyte per milliliter. Following the initial set-up, working solutions 2-5 were immediately introduced into the IAGS from the secondary container. Actual reasons for this disparity remain unconfirmed.

Net filter weights were recorded from pre- and post-sampling weights (Table 3- 17). This measure was an attempt at quality control, but between-test weights were highly variable. Higher filter weights were not unequivocally associated with higher amounts of MDI. For example, FD filters that were sprayed with loading concentration 2 had a net median mass of 107 mg that produced an average amount of MDI equal to 454 ng. The average of these five FD filters yielded a percent recovery of 99.5. On the contrary, the corresponding LD net median mass of 125 mg, which was 18 mg greater than average for FD filters, resulted in 436 ng of MDI with a percent recovery of 96%. Similarly, pipette loading of concentration 2 showed a higher net mass in filters desorbed at the analytical laboratory than desorbed in the field. LD filters outweighed FD filters by 22ng, but both methods produced approximately 93% recover of MDI. Putatively, toluene did not uniformly evaporate.

TABLE 3-17. Net increase in filter mass of technical grade MDI working solutions 1-5 without outliers.

Loading	Working solution	FD net increase in filter mass (± SD)	LD net increase in filter mass (± SD)
Spray	1	48.9 mg (± 8mg)	44.9 mg (± 2mg)
Spray	2	107.6 mg (± 14mg)	121.8 mg (± 13mg)
Spray	3	106.4 mg (± 16mg)	87 mg (± 5mg)
Spray	4	115.6 mg (± 5mg)	82.5 mg (± 3mg)
Spray	5	104.2 mg (± 8mg)	90.6 mg (± 4mg)
Pipette	1	140 mg (± 4mg)	151 mg (± 3mg)
Pipette	2	280 mg (± 4mg)	302 mg (± 4mg)
Pipette	3	268 mg (± 50mg)	301 mg (± 6mg)
Pipette	4	283 mg (± 2mg)	296 mg (± 3mg)
Pipette	5	291 mg (± 3mg)	296 mg (± 3mg)

TABLE 3-18. Net increase in filter mass of technical grade MDI working solutions 3 and 4 with outliers.

Loading	Working solution	LD net increase in filter mass (mg) (± SD)	Net increase filter mass of outlier(mg)	Average of replicates without outlier (mg)
Spray	3	73.1 mg (± 31mg)	17.5	87
Spray	4	78 mg (± 10mg)	60	78

On average, the percent recovery of MDI from pipette loaded filters desorbed in the field and at the analytical laboratory was approximately 92%. The average percent recovery of MDI from LD filters was approximately 93%. In the experiments conducted by the OSHA, LD filters pipette loaded with a stable MDI-urea derivative showed a 97% recovery. The four percent difference was attributed to chemical breakthrough since no difference was observed between FD and LD methods. Since only a 4% difference was observed between filters pipette loaded with pre-derivatized MDI (OSHA) and underivatized-MDI, these results indicated that conditions were optimal for derivatization. However, FD and LD results from pipette loading underivatized MDI still produced significantly different results from theoretical amounts.

To understand the three-way interaction and relative difference between these variables, LSMEANs were compared among loading mechanisms. Based on the average recovery of 93%, results from pipette loading were used as an alternative benchmark to assess spray loaded filters. As shown in Figures 3-5 and 3-6, statistically significant differences were found between pipetting and spraying filters for a fixed desorption method. Only FD filters at workings solutions 1 and 2 showed a significant difference (Figure 3-5). Additionally, LD filters at working solutions 1, and 3-5 exhibited statistically significant LSMEANs between loading mechanisms (Figure 3-6). The significant differences observed at working solution 1 were attributed to evaporation of toluene, which influenced the true concentration of MDI. Accordingly, the theoretical concentration was inaccurate since a recovery greater than 100% was observed.

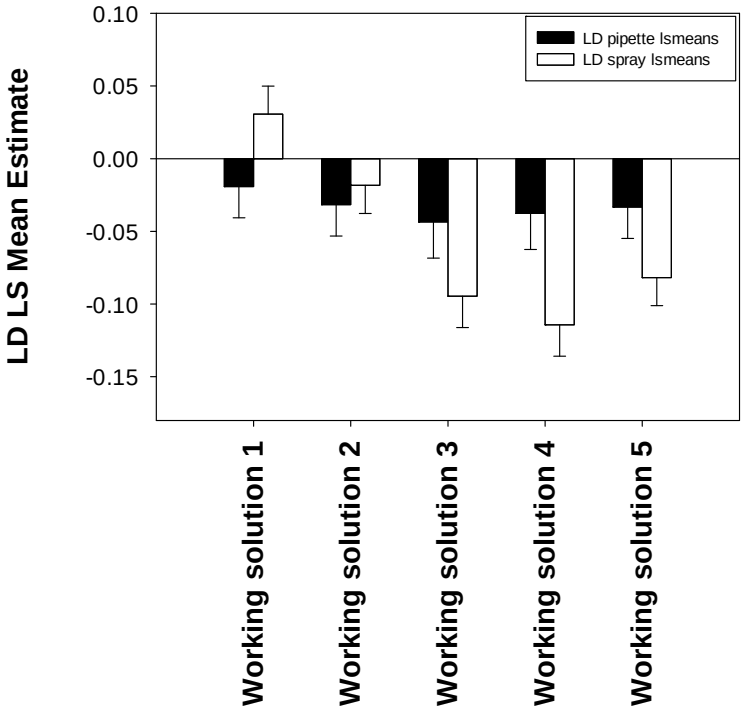

FIGURE 3-6. Comparison of LD LSMEANs from pipette and spray loading filters with technical grade MDI.

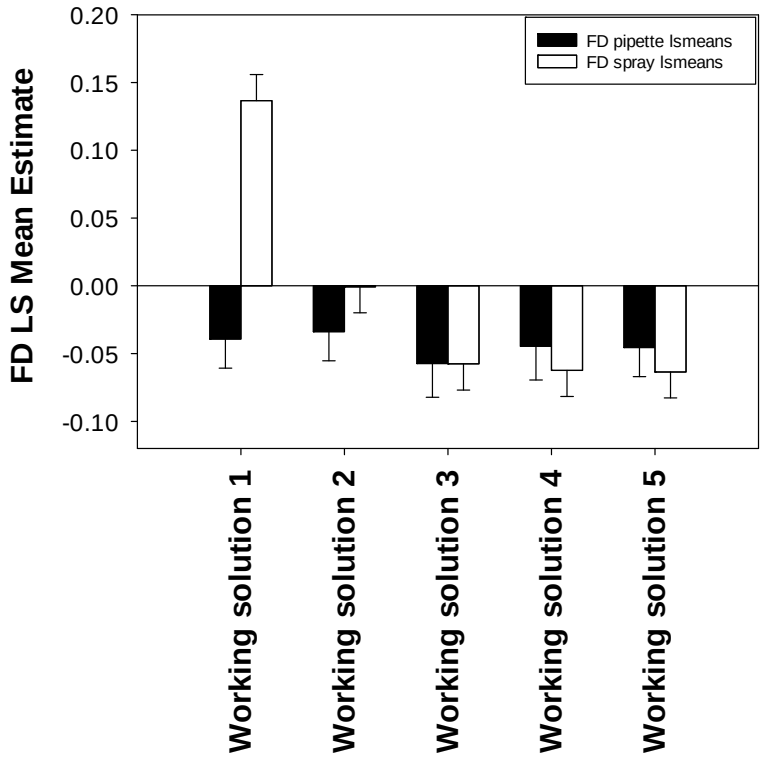

FIGURE 3-7. Comparison of FD LSMEANs from pipette and spray loading technical grade MDI.

LD filters that were spray loaded yielded a significantly lower amount of MDI than spiking a liquid solution, except in working solution 2. There was no significant difference detected in LD filters between loading mechanisms at working solution 2. However, a significant difference between FD filters that were spray and pipette loaded with working solution 2 was observed. There was a 7% difference between the recovered amounts of MDI using FD methods for filters that were spray, or pipette loaded. Chemical breakthrough of the GFF most likely occurred with pipetting, accounting for the lower percent recovery compared to FD and LD filters spray loaded with working solution 2. Percent recoveries from pipette loading were expected to decrease with increasing concentration, not stay the same. Therefore, it is plausible that all breakthroughs of each solution were equal.

Additionally, the count median diamter (CMD) and mass median diameter (MMD) of working solution 2 were 0.41 and 11.4 μm (see Chapter 2). Data from the particles cm^{-3} measured by the Grimm were graphically represented by a unimodal distribution under1 μm. The MMD resembled a toluene particle more than a mixture of toluene and MDI based on density of the components. Therefore, with such a high percentage of counts below 1 μm, and an isocyanate MMD much smaller than 11 μm due to evaporation of toluene (see Chapter 2), it is possible that working solution 2 was better sampled in spray loading consistent with the recommendation of a particle size limit of less than 2 μm. While dried particle sizes were determined in Chapter 2 that illustrated a considerable drop in MMD, oil and ethyl alcohol were used, which changed the behavior of the particle. More research is needed to better characterize true MDI particle sizes emitted from the IAGS. Additionally, characterization of particle sizes was conducted using pure grade and not technical grade MDI. The distribution may change due to the presence of other compounds contained in the formula. Smaller particles and dispersion of MDI may have occurred with the presence of proprietary compounds and other chemical constituents.

Even with the chemical breakthrough, pipette loading achieved desirable recoveries of MDI using both FD and LD methods. However, spray loaded FD and LD filters produced different results when compared to their corresponding pipette loaded filters. While no statistically significant differences were observed between loading mechanisms of FD filters with working solutions 3-5 (Figure 3-6), spray loaded filters desorbed at the analytical laboratory reported a lesser amount of MDI than LD filters spiked with these working solutions (Figure 3-5). These results highlight the interaction between loading, desorption, and concentration, and

the impact on the analytical results, suggesting the importance of desorption method as it relates to airborne particles rather than filters treated with a liquid spike.

For a fixed loading, statistically significant differences were detected between FD and LD LSMEANs when MDI was sprayed onto a filter, but not when it was pipetted. These results were not unexpected as these observations were in good agreement with the findings in side-by-side comparisons in the field (Kaorly and Schaeffer et al.). Loading concentrations 1, 3, and 4 demonstrated statistically significant different LSMEANs (Figure 3-7).

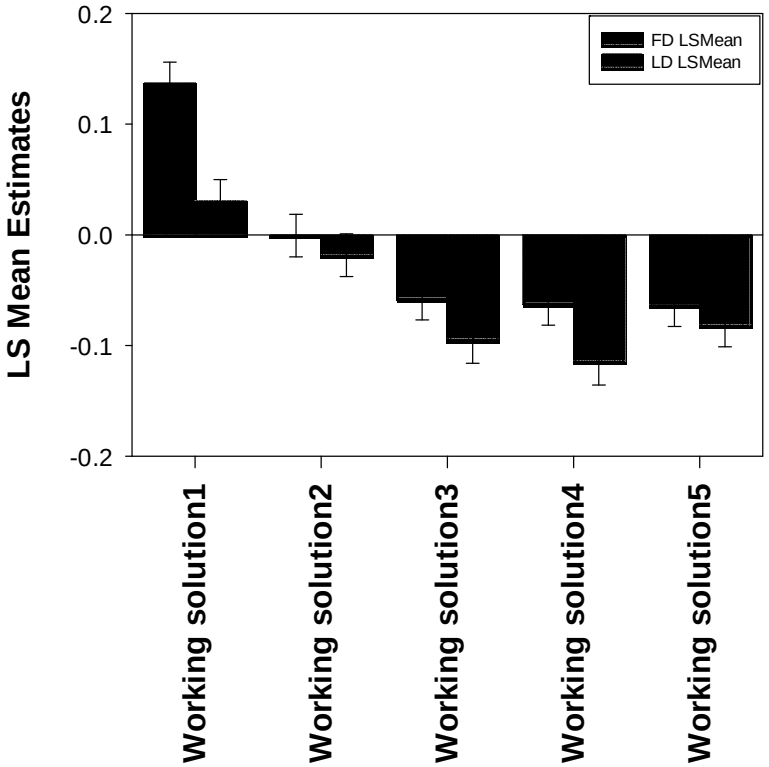

FIGURE 3-8. Comparison of FD and LD LSMEANs from spray loading technical grade MDI.

The true amounts of MDI are shown in Table 3-19 with their corresponding LSMEANs. The underestimation in the FD results corresponding to working solutions 3 and 4 are significantly less than their LD counterpart. These mass differences in working solutions 3 and 4 translate to an 8 and 16 ppb difference between FD and LD samples collected only for 1-minute.

While only a small amount of MDI was applied, a sample air volume of 2 L was used to achieve ppb concentrations germane to real-world observations. Application times of truck bed liners ranged from 5 to 15 minutes. Additionally, early studies of chronic low-level exposures (e.g., 2 ppb) demonstrated the onset of sensitization (Markham and Fishburn 1967). As a result, a difference of 8 ppb may have serious health implications to workers susceptible to sensitization, or already sensitized.

Peak exposures to MDI may be more relevant than lower long-term, or cumulative exposures in terms of risk for developing symptoms of asthma (Allport 2003; Mapp et al. 1988; Mapp et al. 2005; Chan-Yeung and Malo 1995). In working solutions 2-5, LD filters underestimated amounts of MDI that ranged from 20μg to 780μg. An underestimation of 780μg was the equivalent to 80 ppb, which was four-times the ceiling PEL. Depending on the percent of MDI in the product used, process temperature, capture velocity, and air changes per minute during application, airborne concentrations may reach excessive levels, which may even prove fatal if a person is previously sensitized (Chester et al. 2005).

TABLE 3-19. Experimental amounts (average) of technical grade MDI with corresponding LSMEAN with no outliers.

Loading Concentration	Amount of MDI (ng) from FD (LSMEAN)	Amount of MDI (ng) from LD (LSMEAN)
1	318 (0.137)	248 (0.031)
2	454 (-0.0007)	436 (-0.018)
3	1100 (-0.058)	920 (-0.095)
4	2140 (-0.062)	1780 (-0.114)
5	3940 (-0.064)	3780 (-0.082)

The observed statistical significant difference between FD and LD results related to airborne MDI were practically relevant in terms of effect size as described above. These results underscored the challenges of real-world application of these methods for sampling worker

exposure to MDI. While desirable recoveries were attained in the laboratory using a liquid spike of an MDI solution, this approach did not accurately represent the behavior of MDI particles encountered in the field. As mentioned previously, the IAGS may have generated sufficient counts of an optimal particle size (less than 2 μm) upon atomizing working solution 2, facilitating derivatization of MDI in both FD and LD methods. However, derivatization kinetics were significantly altered in spray loading working solutions 3 and 4. While the CMD for these solutions remained at approximately 0.4μm, the MMD of working solution 3 and 4 were 8.8 and 5.9 μm, respectively. This was a reduction in MMD from 11.4 and 13.1 μm observed in MDI working solutions 1 and 2. This reduction in particle size was suggested to be the result of toluene evaporation from the aerosol. The MMDs related to working solutions 3 and 4 were probably closer to the true diameter of MDI with the increase in mass concentration. Therefore, a higher concentration of larger particles (i.e., greater than 2 μm) most likely impacted the filter.

Streicher et al. recommends that GFF methods are used in processes that generate aerosols less than 2 μm(Streicher 1994; Streicher et al. 2000). Isocyanate accessibility to the derivatizing agent is critical when using reagent-coated GFFs, especially in two-component spray applications that consist of an isocyanate and polyol mixture (Booth et al. 2009; Streicher 1994). These products rapidly cure with a half-life of less than two minutes. Micrographs of GFFs containing samples taken during spray applications show minimal contact with the reagent-coated fibers (Bell 1994). As a result, dispersal of the aerosol is negligible, and larger aerosols exhibit an inherent challenge of accessing the reagent (Streicher 1994). Consequently, isocyanates will be lost to competitive reactions within the droplet and underestimated.

Even though particles larger than 2 μm impacted the filters from working solutions 3 and 4, immediate desorption of the filter (i.e., in the field) after sampling was expected to capture the

MDI before consumption to a competitive reaction. While significant differences from the theoretical were determined in filters spray loaded with working solutions 3 and 4, immediate desorption of the filter did minimize the loss of analyte observed in filters desorbed at the analytical laboratory.

While MDI was greatly underestimated from theoretical amounts of working solution 5 by FD and LD methods, no significant difference was observed between desorption methods. Putatively, aggregates of MDI aerosols formed on the filter with this mass concentration of MDI. Theoretical amounts of approximately 4 μg, an amount germane to worker exposures, of MDI were expected to impact the filter. Some dispersion of the aerosolized sample was suggested to occur, facilitated by toluene, which may have been enough to distribute particles from MDI working solutions 2-4 on the filter. However, with the increased mass concentration of working solution 4, agglomeration of aerosols may have started to occur on the filter. The top layer of aerosols may have reacted with the humidity in the air, forming a stable urea, in turn causing a shielding effect of the aerosols beneath it. Theoretically, un-reacted MDI may have been preserved between aerosols that reacted with the 1,2-PP and those that reacted with water. Allport et al. described the reaction between MDI and water to yield polymeric ureas (Allport 2003). The authors suggest that the reaction occurs at the interface with water, and the polyurea produced forms a crust at the surface, hindering diffusion of any chemical species from the reaction. Since diffusion is controlled within this heterogenous interaction, the MDI may be preserved, or unreacted, for a significant amount of time (e.g., up to five weeks has been recroded) (Allport 2003). Therefore, location may not be as important as timing of desorption, especially in atmospheres near the OSHA PEL since exposure to 4 μg of MDI over a 15-min. sampling time yields an airborne concentration of 267 $\mu g/m^3$, which exceeds the PEL. More

research is needed to determine the dynamic behavior of MDI aerosols as they impact a GFF. For example, aggregate formation and temporal relationship may be monitored under a microscope with subsequent gravimetric and chemical analysis.

Underestimations of MDI in both FD and LD filters were attributed to reactions with humidity since air was drawn in via a personal sampling pump. However, the loss of MDI was minimized in FD filters since the extracting solvent dissolved both the derivatizing reagent and any un-reacted isocyanate, allowing the two to combine in solution and form a stable urea-derivative. The LD filters, instead, were not desorbed for at least a few days considering shipping time. MDI aerosols larger than 2 μm may have derivatized only a portion of the aerosol while the un-reacted portion was further exposed to humid air trapped inside the cassette after replacing the top cover and plugs.

Percent Recovery of Pure Grade MDI

In this analysis, the mean FD and LD data were compared to theoretical amounts of pure grade MDI to obtain a percent recovery, or ratio of observed over theoretical. A ratio of one represented a 100% recovery of MDI. The least squares means (LSMEAN) were calculated (Table 3-20 and 3-21) to integrate the effects of desorption method, loading mechanism, and loading concentration. Indoor air quality, specifically temperature and humidity, was recorded during this analysis using a TSI Q-Trak (Table 3- 22).

TABLE 3-20. LSMEANs associated with pipette loading pure grade MDI

Loading	Desorption	Concentration	Log 10_ratio LSMEAN	Pr> |t|	Percent recovery
Pipette	Field	6	-0.04793	0.0001	89.6%
Pipette	Field	7	-0.1175	<0.0001	76.3%
Pipette	Field	8	-0.1226	<0.0001	75.4%
Pipette	Field	9	-0.1112	<0.0001	77.4%
Pipette	Field	10	-0.1998	<0.0001	63.1%
Pipette	Laboratory	6	-0.04453	0.0003	90.3%
Pipette	Laboratory	7	-0.1111	<0.0001	77.4%
Pipette	Laboratory	8	-0.1271	<0.0001	74.6%
Pipette	Laboratory	9	-0.1055	<0.0001	78.4%
Pipette	Laboratory	10	-0.2131	<0.0001	61.2%

TABLE 3-21. LSMEANs associated with spray loading pure grade MDI

Loading	Desorption	Concentration	Log 10_ratio LSMEAN	Pr> |t|	Percent recovery
Spray	Field	6	-0.01736	0.1505	96.5%
Spray	Field	7	-0.1379	<0.0001	73.3%
Spray	Field	8	-0.1065	<0.0001	78.7%
Spray	Field	9	-0.07391	<0.0001	84.4%
Spray	Field	10	-0.1510	<0.0001	70.8%
Spray	Laboratory	6	-0.1062	<0.0001	78.5%
Spray	Laboratory	7	-0.1270	<0.0001	74.8%
Spray	Laboratory	8	-0.1216	<0.0001	75.6%
Spray	Laboratory	9	-0.08183	<0.0001	82.9%
Spray	Laboratory	10	-0.1836	<0.0001	65.5%

TABLE 3-22. Humidity and temperature of laboratory on both sampling days

	Loading	Desorption	Concentration	Humidity (average)	Temperature (average)
Day 1	Spray	Field	6	20.4%	68.8 °F
	Spray	Field	7	20.4%	68.8 °F
	Spray	Field	8	20.4%	68.8 °F
Day 2	Spray	Field	9	9.1%	72 °F
	Spray	Field	10	9.1%	72 °F

Spray and Pipette Loading Pure Grade MDI

A total of 107 observations were collected in this evaluation using pure grade MDI. As defined in the Methods section, filters were pipette loaded (n=50) and spray loaded (n=57). Data were log-transformed to make inferences using a ratio of observed over theoretical. Normality and independence of data were observed in a studentized residual versus predicted plot, illustrated in Figure 3-9. No outliers were observed in this data set that corresponded to a delivery or measurement error.

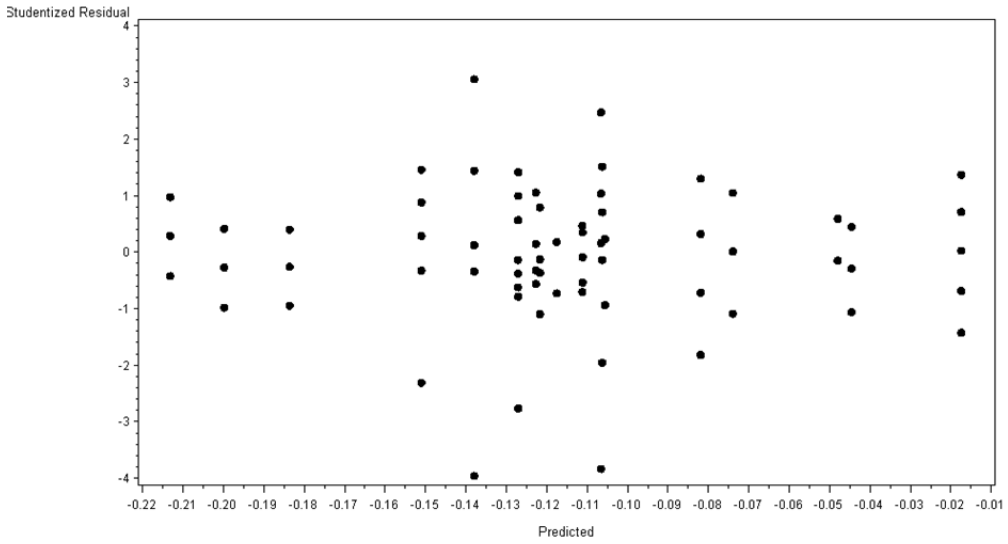

FIGURE 3-9. Studentized residual versus predicted plot of pure grade MDI data.

This data set was unbalanced due to seven extra IAGS samples (LD=6 samples; FD=1 sample) compared to samples collected from pipette loading. Using the Mixed Procedure in SAS, which is a generalization of the GLM, Type III Tests for fixed effects were computed to evaluate the relationships between loading mechanism, desorption method, and loading concentration. The output of inferential statistics from the Mixed Procedure was assumed to be the same as if the GLM procedure was invoked; however, the scope was broader, allowing for analysis of several sources of variation. For example, analysis of simple effects was accomplished by the SLICE statement within the Mixed Procedure, which provided a general

mechanism for performing a partitioned analysis of the LSMEANs for an interaction. LSMEANs were computed for each effect, and used to make comparisons on interactions and main effects.

As expected, a statistically significant ($p<0.05$) three-way interaction was determined (Table 3-23), indicating that analytical results varied with the effects of loading concentration, desorption method, and loading mechanism. Therefore, interpretations of these interactions supplant any further analysis of the main effects or two-way interactions. As shown in Table 3-23, the F Value was reported instead of the Type I and III SS like in Table 3-14. Type III Tests were computed only within this mixed linear model.

TABLE 3-23. Iterations of two-way and three-way interactions and results of pure grade MDI.

Interaction	Degrees of Freedom	Type III Tests F Value	Pr > F
Loading*Desorption	1	6.27	0.0141
Loading *Concentration	4	4.97	0.0260
Desorption*Concentration	4	2.91	0.0012
Loading*Desorption*Concentration	4	2.56	0.0441

The three-way interaction included two different two-way interactions with crossovers (Figures 3-10 and 3-11). A change in pattern was observed in Figure 3-10 and 3-11, but the change in the two-way interaction between loading mechanism and desorption method was not equal as loading concentration increased. The effect of concentration was much greater in FD and LD filters spray loaded with MDI. These observations are in good agreement with results from the technical grade MDI comparison between loading mechanisms, desorption method, and loading concentrations.

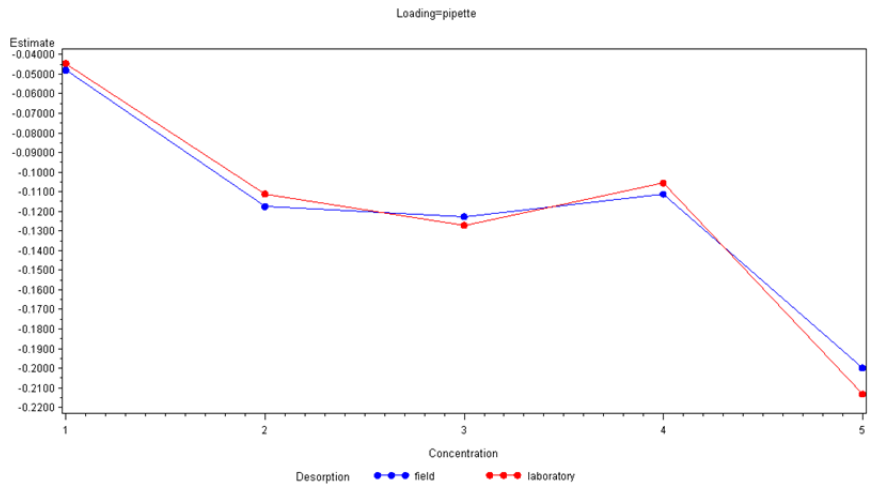

FIGURE 3-10. Three-way interaction of pipette loading pure grade MDI.

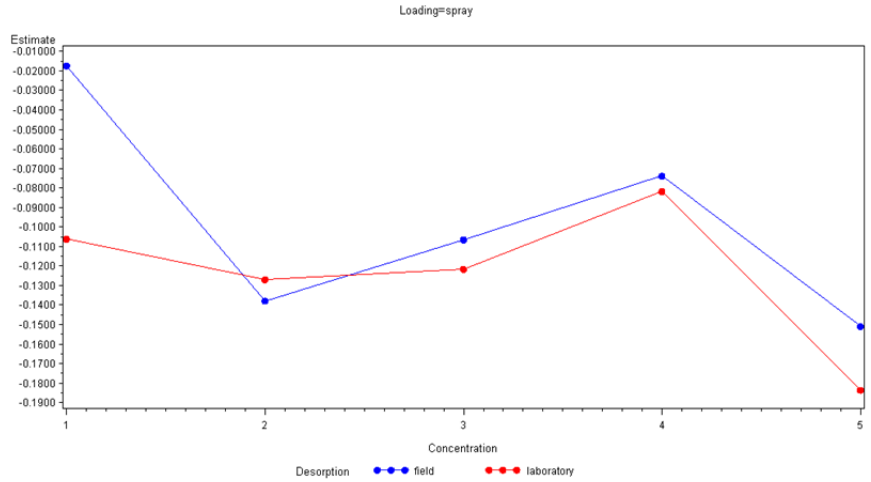

FIGURE 3-11. Three-way interaction of spray loading technical grade MDI.

Table 3-20 presents LSMEANs from pipette loading FD and LD filters with loading concentrations 1-5. At each level, the LSMEAN underestimated the expected amount of MDI. The test statistic associated with each observation indicated all pipette-loading samples were statistically significant ($p<0.05$). Unlike technical grade MDI, pure MDI solutions did not contain excipient compounds, which should have facilitated the stabilization of MDI by its reaction with 1,2-PP. However, differences between observed to theoretical MDI ranged from approximately 26 to 1600 ng in working solutions 1-5 (Table 3-21). This range of underestimations was much wider than technical grade MDI, which was 12-500 ng. These results

suggest that quantitative determinations of pure grade MDI were further underestimated from the theoretical compared to technical grade, even under optimal conditions for derivatization provided by liquid spiking the working solutions of MDI.

As shown in Table 3-24, the LD mean mass collected from working solution 6 resulted in the smallest deviation from the theoretical while LD filters loaded with working solution 10 produced the largest percent error (theoretical-observed/theoretical *100). The LD LSMEANs in working solutions 6 and 10 were -0.044 and -0.21 (Table 3- 20). Unlike in technical grade MDI, the LD LSMEAN in working solution 1 did reveal a statistically significant difference from zero. All LSMEANs associated with each desorption method and working solution showed a statistically significant difference (Table 3-20). The average LD mass from working solution 6 was 244 ng while the average mass from LD filters loaded with working solution 1 (corresponding mass concentration in technical grade MDI) was 278 ng. Additionally, the average LD mass from working solution 10 was 2560 ng while the average mass from working solution 5 (corresponding mass concentration in technical grade MDI) was 4575 ng. Since these two grades of MDI were not exactly the same mass concentration, a mass percent difference was not calculated; however, examining the differences in percent recovery between these two grades further demonstrated the magnitude of underestimation of pure grade MDI.

TABLE 3-24. Mass differences between observed to theoretical amounts of pipette loaded with pure grade MDI .

Working solution	Theoretical amount of MDI	FD difference from theoretical	LD difference from theoretical
6	270.2	-28.2 ng	-26.2ng
7	532	-126 ng	-120 ng
8	1026	-252 ng	-260 ng
9	2014	-454 ng	-434 ng
10	4180	-1540 ng	-1620 ng

No significant differences were found between FD and LD methods at each loading concentration for a fixed loading mechanism, which agrees with the results from pipette loading technical grade MDI. The greatest difference between FD and LD LSMEANs was detected in working solution 10. This difference resulted in an estimate of 0.01337, which correlated to a mass of 80 ng. A difference of 2 ng was observed in working solution 1.

The overall average percent recovery and standard deviation for FD and LD filters was 76.3% ± 10% and 76.4% ± 9%, respectively. The average between FD and LD methods in working solution 1 demonstrated a percent recovery of approximately 90%. Working solution 7 showed a large decrease, approximately 13%, from working solution 6. Percent recovery appeared to stabilize at working solutions 7-9 with an average of approximately 76% recovery. However, working solution 10 exhibited only a 60% recovery of MDI, which was another 13% drop in percent recovery. These differences showed a greater underestimation of MDI than filters pipette loaded with technical grade MDI.

Liquid spiking filters with working solutions of MDI was expected to facilitate distribution of MDI across the filter, and consequently derivatization with 1,2-PP in both FD and LD methods. While no discernable difference was observed between analytical results from each desorption method, pipette loading of underivatized pure grade MDI did not achieve ideal percent recoveries seen in the OSHA evaluation using pre-derivatized MDI. A caveat to delivering liquid solutions was chemical breakthrough since GFFs were not developed for such loading. The large decrease from 90 to 70% recovery observed in working solutions 6 and 7 was most likely due to the increase in pipette volume. Not as much breakthrough may have occurred when a filter was challenged with 0.193 ml of working solution 6 compared to 0.380 ml of

working solution 7-10. Further research is needed with lower pipette volumes of underivatized MDI to determine the impact of breakthrough on quantitative determinations.

As shown in Table 3-21, significant differences from zero were further observed in FD and LD LSMEANs related to spray loaded filters with pure grade MDI working solutions 6-10. As with technical grade MDI, underestimations were not unexpected; however, pure grade MDI was conjectured to yield higher amounts of MDI consistent with the absence of excipient compounds that were present in technical grade MDI. These compounds were anticipated to interfere with the derivatization of MDI. Instead, percent recoveries of pure grade were much lower than those observed in technical grade MDI (see *Comparison between technical and pure grade MDI*). However, the FD LSMEAN specific to working solution 1 was -0.01736, which was the closest estimate to zero in the data set. This LSMEAN corresponded to a quantitated amount of 260 ng of MDI, which was 10 ng less than the theoretical (i.e., 270.2ng). The equivalent LD LSMEAN was -0.1062, deviating significantly from zero. The average amount of MDI quantitated from LD filters loaded with working solution 6 was 212 ng. A significant difference was demonstrated in the comparison between FD and LD methods at working solution 6, indicating that while both methods underestimated MDI at concentration 6, filters desorbed at the analytical laboratory were less accurate than FD methods.

Working solution 6 was comparable to the mass concentration of working solution 1 (technical grade MDI), confirmed by HPLC at the WOHL. The negative LSMEANs associated with working solution 6, consistent with underestimation of MDI, were significantly different than the LSMEANs specific to the interaction of effects observed in working solution 1. While the results from working solution 6 did not confirm the reason for disparity (i.e., greater than 100% recovery) in working solution 1, alteration of the mass concentration was more plausible.

However, pure grade MDI quantitations as compared to the theoretical were much lower than technical grade MDI quantitations in both FD and LD filters.

Comparisons between FD and LD LSMEANs obtained from working solution 10 represented a difference of 0.03264, which generated a p-value of 0.057. According to the alpha used in this study, this difference is most likely due to chance; however, since the statistic is so close to the alpha, this difference should be considered important. In terms of amount collected, a difference of 220ng was detected between FD and LD methods. A difference of this magnitude is still concerning, especially if sensitized individuals are being monitored. Sampling methods need to be accurate to ensure the health and safety of the worker.

No significant differences were observed between the remaining FD and LD LSMEANs (i.e., from concentrations 7-9). The differences between FD and LD methods at concentrations 2-4 ranged from 20ng to 60ng.

Like the interpretation of technical grade MDI results, underestimations of atomized pure grade MDI were attributed to aerosol particle sizes. Briefly, the reported MMDs in working solutions 1 and 2 were suggested to be more representative of a toluene aerosol than MDI. Following evaporation of toluene, a much smaller MDI particle would be left attached to the GFF. Conversely, the MMD of working solutions 3-5 resembled an MDI aerosol more closely than working solutions 1 and 2 since mass concentration increased. Toluene likely evaporated at a much faster rate in working solutions 3-5 based on the Kelvin effect (see Chapter 2). Assuming the median of the particle size distribution is less than 2 μm in working solution 6 and 7, similar results as technical grade working solution 2 should have been attained. However, both FD and LD samples loaded with aerosolized MDI solution 7 were both significantly different than the theoretical. Both sets of filters loaded with working solution 7 reported over 96% recovery of

technical grade MDI, which was not considered a statistically significant difference from the theoretical. Therefore, another variable affecting or competing with derivatization was present.

As stated earlier, pure grade MDI did not contain proprietary compounds, or other forms of MDI that were present in the technical grade MDI. The technical grade MDI was a customized formula of chemicals used to produce a type of polyurethane with specific properties to meet certain product specifications. This blend of chemicals either partially (since significant underestimations were also observed in technical grade MDI) insulated the MDI from nucleophilic agents, or the modified MDI present may have augmented the signal for monomeric MDI during analysis. Accordingly, pure grade MDI impacting the filter is more exposed than technical grade to nucleophilic agents present in the atmosphere. Only FD samples of work solution 6 were not statistically significant from the theoretical. All other IAGS collected samples were statistically different than the theoretical. Results from working solution 6 highlights the importance of immediate desorption of the filter since there was a significant difference between these FD and LD samples. Additionally, at the time the MDI working solutions 6-8 were aerosolized, the relative humidity (RH%) in the laboratory was 20.4%. Comparatively, the RH% during the time working solutions 9 and 10 were aerosolized was 9.1%. The two lowest percent recoveries of MDI were observed in LD filters treated with working solution 7 and 10. These filters were collected on separate days with two different humidity levels present. Even though the relative percent humidity on day 1 was two times that on day 2, working solution 10 still yielded a lesser amount of recovered MDI. More research is needed to determine the effects of the simultaneous collection of MDI and humidity on a filter. Revising the volume delivered from the pipettes to ensure no breakthrough, a comprehensive model simulating a variety of RH% and temperatures while leveraging the successes from this

current study may attenuate some of the limitations of this study and further knowledge in sampling MDI. Furthermore, micrographs of filters after collection of aerosolized MDI from each working solution would provide knowledge on the formation of agglomerates (or deformation or shattering of aerosols) related to concentration and impaction on the filter, as well as the effects of humidity and agglomerates on analytical results.

Comparison between Technical and Pure Grade MDI

Using the Mixed Procedure in the SAS System, a common model four-way ANOVA was performed to compare pure grade and technical grade MDI across the three variables: loading mechanism, desorption method, and loading concentration. A model-based fixed effects SE method was used with residual degrees of freedom method to make comparisons of 191 observations. Normality and independence of the data was determined using a plot of studentized residuals versus predicted, as well as a quantiles plot and histogram, for the log-based ratio calculated for technical-, and pure grade MDI (Figure 3-12).

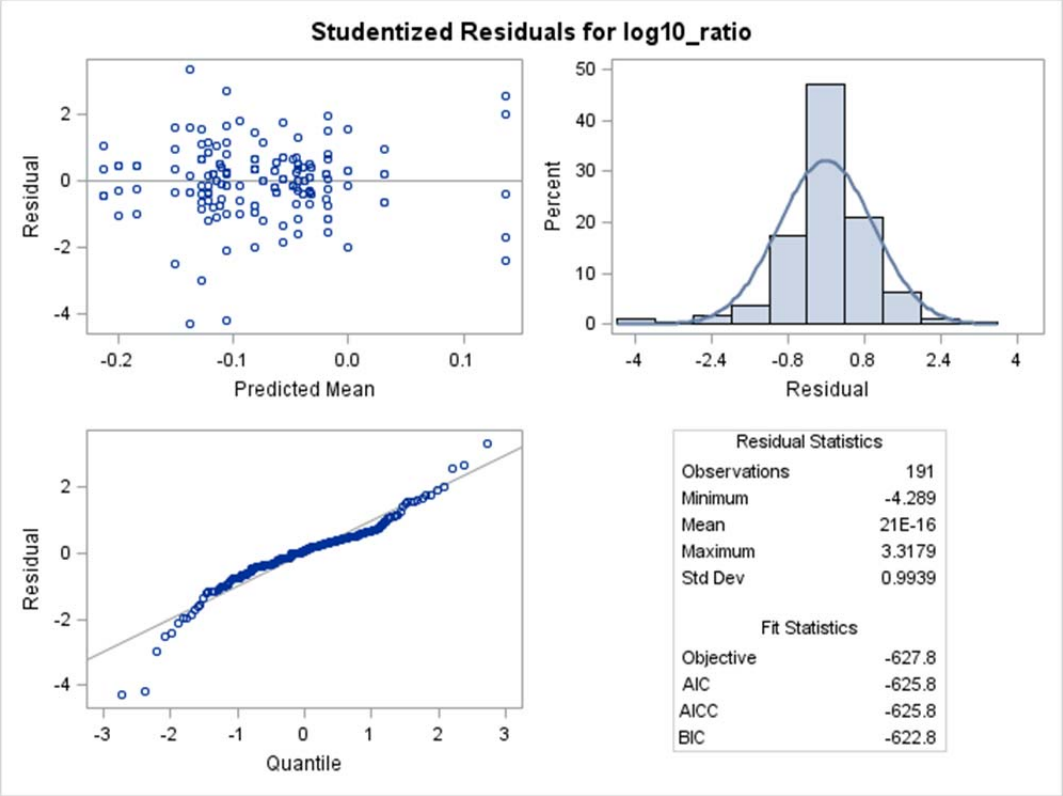

FIGURE 3-12. Studentized residuals for log-based ratios of both technical and pure grade MDI.

The Mixed Procedure demonstrated the presence of significant interactions ($p<0.05$), including two- and three-way interactions between variables (Table 3-25); however, no four-way interaction was observed. Estimates from simple differences (or partitioned analysis) of the LSMEANs from all four variables were used to evaluate statistical significance (Table 3-26 and 3-27).

TABLE 3-25. Type 3 Tests of Fixed Effects from Mixed Procedure comparing technical and pure grade MDI.

Effect	Degrees of Freedom	Type III Tests F Value	Pr > F
Grade	1	476.67	<.0001
Desorption	1	18.19	<.0001
Grade*Desorption	1	0.29	0.5885
Loading	1	4.40	0.0376
Grade*Loading	1	0.21	0.6475
Desorption*Loading	1	32.56	<.0001
Grade*Desorption*Loading	1	4.48	0.0359
Concentration	4	95.99	<.0001
Grade*Concentration	4	23.51	<.0001
Desorption*Concentration	4	4.07	0.0037
Grade*Desorption*Concentration	4	0.99	0.4123
Loading*Concentration	4	8.39	<.0001
Grade*Loading*Concentration	4	29.28	<.0001
Desorption*Loading*Concentration	4	5.97	0.0002
Grade*Desorption*Loading* Concentration	4	0.17	0.9525

TABLE 3-26. Estimates from simple difference LSMEANs associated with pipette loading technical and pure grade MDI

Loading	Desorption method	Technical MDI working solution	Pure MDI working soltuion	LSMEAN estimate	Pr> |t|
Pipette	Field	1	6	-0.00870	0.6001
Pipette	Field	2	7	-0.08369	<0.0001
Pipette	Field	3	8	-0.06529	0.0004
Pipette	Field	4	9	-0.06654	0.0003
Pipette	Field	5	10	-0.1543	<0.0001
Pipette	Laboratory	1	6	-0.02534	0.1279
Pipette	Laboratory	2	7	-0.07941	<0.0001
Pipette	Laboratory	3	8	-0.08353	<0.0001
Pipette	Laboratory	4	9	-0.06795	0.0002
Pipette	Laboratory	5	10	-0.1798	<0.0001

TABLE 3-27. Estimates from simple difference LSMEANs associated with spray loading technical and pure grade MDI

Loading	Desorption method	Technical MDI working solution	Pure MDI working soltuion	LSMEAN estimate	Pr> | t |
Spray	Field	1	6	-0.1540	<0.0001
Spray	Field	2	7	-0.1372	<0.0001
Spray	Field	3	8	-0.04890	0.0021
Spray	Field	4	9	-0.01152	0.4619
Spray	Field	5	10	-0.08747	<0.0001
Spray	Laboratory	1	6	-0.1369	<0.0001
Spray	Laboratory	2	7	-0.1086	<0.0001
Spray	Laboratory	3	8	-0.02705	0.1044
Spray	Laboratory	4	9	0.03241	0.0279
Spray	Laboratory	5	10	-0.1018	<0.0001

As shown in Table 3-26, no significant difference was observed in filters that were pipette loaded with working solutions 1 and 6. These working solutions correspond to technical (working solution 1) and pure grade (working solution 6) MDI solutions that contained comparable mass concentrations of MDI. Log-based ratios were analyzed using the class information depicted in Table 3-28.

TABLE 3-28. Class level information in the statistical model comparing technical and pure grade MDI

Class	Levels	Values
Grade	2	Pure and Technical
Desorption	2	Field and Laboratory
Loading	2	Pipette and Spray
Working Solution	5	1 2 3 4 5

FD and LD filters pipette loaded with technical and pure grade MDI solutions 1 and 6 produced a narrow range of percent recoveries when analyzed separately. For example, a 91.4% recovery was reported for FD filters while LD filters demonstrated a slight increase 95.7%. Additionally, the percent recovery of technical grade MDI was 89.6% and 90.3% in FD and LD filters, respectively. The lack of statistical significance between the LSMEAN estimates

associated with these percent recoveries was in agreement with the interpretation of chemical breakthrough.

A statistically significant difference was observed between pure- and technical grade MDI in FD and LD filters that were pipette loaded with working solutions 2-5. Statistical significance was expected between technical and pure grade MDI since a steep decrease in percent recovery occurred in filters liquid spiked with loading concentrations 7-10.

Liquid spiking filters with working solutions of MDI was expected to facilitate distribution of MDI across the filter, and consequently derivatization with 1,2-PP in both FD and LD methods. While consistent percent recoveries of technical grade MDI were reported, a large decrease in pure grade MDI was observed between working solution 6 and 7. The working solutions were undersaturated, with toluene in excess of MDI. The hydrophobic aromatic rings of MDI, as well as the NCO functional groups, were solvated with toluene. Toluene breakthrough was volume dependent. An increase from 0.193 ml to 0.380 ml exceeded the capacity of the filter. The increase in pipette volume caused toluene to breakthrough the filter, which rendered a loss of analyte since the MDI traveled with the solvent. As a result, filters pipette loaded with pure grade MDI significantly underestimated MDI as compared to both the theoretical and technical grade MDI. The presence of other compounds in technical grade MDI may have minimized the loss with chemical breakthrough, or augmented the signal of monomeric MDI (e.g., modified MDI may co-elute with monomeric MDI). The NCO group in technical grade MDI may have been less solvated due to the presence of other hydrophobic compounds. Accordingly, the NCO group was able to access 1,2-PP instead of following toluene through the filter. Without knowing the identity or chemical and physical properties of these proprietary compounds, the discrepancy in analytical results between technical and pure grade

MDI remains unclear. Using other nonpolar organic solvents, and adjusting the pipette volume will form the focus of a future study.

As shown in Table 3-27, statistically significant differences were further observed between technical and pure grade MDI in the IAGS data. Results from the FD filters treated with pure grade MDI showed a significant difference from technical grade MDI at each concentration except working solution 4 and 9. The percent recoveries of these technical and pure grade MDI working solutions were 86.6 and 84.4%, respectively, when analyzed separately. The simple difference LSMEANs was -0.01152, which was the smallest estimate of simple difference LSMEANs observed in both the pipette and IAGS comparison (Tables 3-26 and 3-27). The outcome of 84.4% from the individual analysis of the pure grade model at working solution 9 marked an approximate 6% increase from working solution 8. Subsequently, the percent recovery of working solution 10 exhibited a significant decrease to 70.8%. A similar waxing and waning effect was observed in the LD samples.

Simple difference LD LSMEANs in the IAGS data exhibited a statistically significant difference except between working solutions 3 and 8. This comparison between technical and pure grade MDI yielded an LSMEAN estimate of -0.02705, which was not a large enough difference to be significant. When analyzed separately, the LD method yielded a percent recovery of 80.5 and 75.6% following treatment with technical and pure grade MDI working solutions 3 and 8, respectively. The lack of significance in the simple difference LSMEAN between these two grades of MDI suggested an effect size of at least 4.9% recovery. Additionally, the comparison of technical and pure grade MDI working solutions 4 and 9, respectively, produced an LSMEAN of 0.03241, which was the only positive LSMEAN in this

comparison. LD filters treated with pure grade MDI working solution 9 yielded a higher percent recovery than technical grade MDI working solution 4.

Comparison of the net increase in filter mass after IAGS treatment with technical and pure grade MDI did not account for the large difference observed between these two grades (Table 3-29). For example, the average net increase in FD filters was 104.2 and 119.8 mg after collection of technical and pure grade working solutions 5 and 10, respectively. Even though pure grade filters showed a higher net amount (i.e., 15 mg), the percent recovery of working solution 10 was only 70%, which was 16% less than the percent recovery observed from the corresponding technical grade working solution. Additionally, a 30 mg difference between FD and LD filters was observed at working solution 10, which was one of the largest differences observed in both data sets of MDI. However, a significant difference was not detected between FD and LD methods despite this discrepancy. This result further substantiates that filter mass was not an accurate indicator of reproducibility between data sets. Evaporation or polymerization of the isocyanate (depending on its accessibility to the reagent) occurred at different rates. Despite this limitation in quality control, results from the IAGS validation suggested that the system performed at a high level, consistent with design values.

Whether differences between technical and pure grade were significant or not, these results were practically relevant with serious worker implications since accuracy of MDI collection and analysis was related to composition of the product. An effect size greater than 4.9% (i.e., observed between working solutions 4 and 9) was still an important finding since this may correlate with a considerable amount of MDI mass depending on concentration encountered. As stated earlier, chronic low-level exposures (e.g., 2 ppb) demonstrated the onset of sensitization (Markham and Fishburn 1967). A difference between these two grades of MDI

warrants further research to determine how much composition influences collection of MDI using GFF methods.

TABLE 3-29. Net increase in filter mass with pure grade MDI.

Loading	Concentration	FD net increase in filter mass (± SD)	LD net increase in filter mass (± SD)
Spray	6	37.2 mg (± 9 mg)	34.1 mg (± 7 mg)
Spray	7	107.4 mg (± 16 mg)	95.9 mg (± 9 mg)
Spray	8	112.7 mg (± 7 mg)	116.7 mg (± 8 mg)
Spray	9	109.2 mg (± 8 mg)	107.7 mg (± 4 mg)
Spray	10	119.8 mg (± 7 mg)	90.6 mg (± 4 mg)
Pipette	6	163.3 mg (± 2 mg)	162.4 mg (± 1 mg)
Pipette	7	321.9 mg (± 4 mg)	320.6 mg (± 2 mg)
Pipette	8	326.9 mg (± 6 mg)	322.7 mg (± 2 mg)
Pipette	9	325.1mg (± 2 mg)	321.5 mg (± 2 mg)
Pipette	10	322.4mg (± 3mg)	348.5 mg (± 6 mg)

The presence of other compounds in the technical grade MDI may account for these observed differences. Specifically, a bulk sample of STBL product was obtained from a local company that contained a proprietary component (25-45% w/w), and modified MDI (less than 10% w/w). The proprietary component may have insulated the MDI from competitive reactions; however, this would not explain the difference between FD and LD determined in this study. Therefore, it is likely that this component only slowed down the reaction kinetics so that upon field desorption, more MDI was collected while the aggregate MDI on LD filters reacted with humidity in the air. Since not all technical grade loading concentrations exhibited a statistically significant difference that were spray loaded, it is plausible that depending on amount of derivatizing agent in the vicinity of impaction, as well as the amount of MDI accessible within this aerosol mixture to 1,2-PP made a significant difference. When pure MDI was spray loaded, no significant difference was observed between desorption methods, but both methods underestimated MDI even more than technical grade. Toluene was the only other compound

present in pure MDI solutions, and as soon as toluene evaporated, a layer of pure MDI was in direct contact with the air unlike the technical grade. Additionally, technical grade MDI contained modified MDI in addition to the monomer. The analytical laboratory did not distinguish between modified MDI and monomeric MDI. Therefore, signal or peak amplification of monomeric MDI may have occurred if these two compounds co-elute. Accordingly, analytical results of technical grade MDI may reflect a combined amount of monomeric and modified MDI.

CONCLUSION

The goal of this research was to determine the accuracy of FD and LD methods using a basic model of pipette and spray loading MDI. For example, MDI was neither mixed with a polyol, nor heated. The difficulties and challenges of sampling MDI were demonstrated in the results from a controlled setting. A significant difference was observed not only between FD and LD filters treated with MDI using the IAGS, but between grades of MDI, as well. A three-way interaction was present that demonstrated how the effect of concentration on quantitative determinations differs with desorption method and depends on loading mechanism. Alternatively, a significant difference was observed between spraying and pipetting FD and LD filters at different concentrations. While a 97% recovery of pre-derivatized MDI was produced in the OSHA evaluation, these results do not accurately reflect the influence of competitive reactions and the impact of aerosol size on quantitation of field samples.

Overall, this research showed that filters treated with aerosolized MDI and then immediately desorbed in the field were more accurate than filters that were shipped and desorbed at the analytical laboratory. However, FD filters significantly underestimated expected amounts

of MDI, which has broad implications in prudent practices for sampling worker exposures to MDI.

Many limitations inherent to this study suggest avenues for continued research. For example, one limitation of this study was the potential chemical breakthrough of MDI through the sampling filter. In addition, the particle size measurements did not reflect dried MDI particles, but rather particle diameters of an intermediate stage of the aerosol. Additional information on the particle size distribution of airborne MDI (after evaporation of toluene) will provide more evidence on the derivatization efficiency related to particle size. Finally, without controlling temperature and humidity in this study, the effect of simultaneous collection of water vapor remains equivocal. An experimental matrix of different test conditions of humidity will allow determination of the rates of competitive reactions and their impact on analytical results.

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CHAPTER 4—ATOMIZATION OF PURE GRADE TOLUENE DIISOCYANATE: A LABORATORY EVALUATION OF TWO SAMPLING METHODOLOGIES FOR MONITORING WORKER EXPOSURES USING A NOVEL SPRAY-ON DELIVERY SYSTEM

SUMMARY

The goal of this research was to determine the suitability of immediate desorption of filters treated with aerosolized pure grade toluene diisocyanate. Using an isocyanate aerosol generating system, analytical results from immediate filter desorption were compared to desorption at the analytical laboratory. Five working solutions of toluene diisocyanate were prepared with toluene to determine if concentration linearly predicted the percent recovery of toluene diisocyanate . A total of 50 samples were collected using field and laboratory desorption methods. Data were log-transformed to make inferences using a ratio of observed over theoretical. Data were balanced indicating that the factors in this model were orthogonal. Using the general linear model, the omnibus test of the model showed a significant F-value of 5.26 (p-value <0.0001), the main effect of desorption alone was not found to predict percent recovery with an F-value of 1.26. The F-value associated with concentration was 9.34, which produced a p-value < 0.0001. Concentration was linearly related to percent recovery after accounting for desorption. Significant two-way interactions between desorption and concentration was not observed. Generally, analytical results from each loading concentration of toluene diisocyanate did not vary with desorption method. Differences between field and laboratory desorption sampling methods were not significant following sample collection of atomized working solutions 2-5. However, a significant difference in percent recovery between desorption methods was observed at working solution 1, which emphasizes the importance of immediate desorption for a specific concentration of toluene diisocyanate composed of a particular fraction of gas and aerosol phases. Field and laboratory desorbed filters treated with atomized toluene diisocyanate

demonstrated a statistically significant underestimation of recovered toluene diisocyanate compared to the theoretical amount across all working solutions. The percent recovery profile of atomized toluene diisocyanate was similar to the pure grade methylene bisphenyl isocyanate results presented in Chapter 3. Loss of isocyanate to competitive reactions with water vapor most likely accounts for the low percent recovery observed in both this study and the methylene bisphenyl isocyanate study. While volatilization may exclusively account for the loss of isocyanate (i.e., to the inside walls of the cassette), methylene bisphenyl isocyanate has a much lower vapor pressure than toluene diisocyanate, and was not anticipated to volatilize during that study. As water vapor from ambient air was drawn onto the filter by the sampling pump, either hydrolysis of the isocyanate to its respective diamine occurred, or a polymeric urea was formed. As the concentration of TDI increased, the particle size distribution increased as well, causing agglomeration and less contact with the derivatizing agent. In general, immediate desorption of filters treated with aerosolized TDI did not enhance percent recovery.

INTRODUCTION

Toluene diisocyanate (TDI) is an industrially important cross-linking agent used extensively to manufacture a wide range of end-use polyurethane products (Ruwona et al. 2010; Wisnewski, Hettick, and Siegel 2011; Hettick et al. 2012; Broberg et al. 2008{Allport, 2003 #37; Allport 2003). Otto Bayer first described the versatile properties of TDI-derived polyurethane-based materials in 1937 (Ulrich 1996; Henneken, Vogel, and Karst 2007; Cummings and Booth 2002). With the presence of two isocyanate (NCO) functional groups, TDI exhibits strong chemical reactivity that is capable of polymerization (Broberg et al. 2008{Raulf-Heimsoth, 1998 #84)}. Globally, TDI is the second most abundantly produced diisocyanate (Wisnewski, Hettick, and Siegel 2011); both TDI and methylene bisphenyl isocyanate (MDI) account for over 90% of

the diisocyanate market (Hettick et al. 2012; Allport 2003). Predominantly, the greatest use of TDI is in the flexible slabstock industry to produce bedding and furniture (e.g., polyurethane foam mattress, and carpet backing) (Cummings and Booth 2002{Ulrich, 1996 #120).

Other applications of TDI can be found in other sectors, such as consumer / do-it-yourself adhesives, sealants and coatings, construction, automotive, painting, manufacturing of plastic auto parts, packaging, and roofing, and mining (Lofgren et al. 2003; Agency 2011). While variations of TDI-derived polyurethane products exist with different specifications and properties (e.g., elasticity, softness, density, compression performance, flame resistance), open-cell flexible foam is the largest application (Ulrich 1996). Other chemicals (e.g., polyol, water, surfactant, and catalyst) are also added to the reaction, which serve as blowing agents, chain extenders, and cross-linking agents. About 15-35% of the polymer synthesized in this reaction is a result of TDI reacting with water. Hot molded flexible polyurethanes derived from TDI are used throughout the upholstery of automobiles (e.g., seating, instrument panels, head rests, arm rests) (Ulrich 1996).

The term TDI is non-specific, which is used to describe all types of TDI-related species (e.g., isomers and modified TDI) (Allport 2003). Manufacturing of TDI involves dinitration of toluene in a two-stage process that yields an 80/20 mixture of 2,4- and 2,6-TDI. This mixture is 99% monomeric TDI (Allport 2003). While 80/20 TDI is the predominant reaction product, a 65/35 mixture of 2,4- and 2,6-isomers may also be produced by separation of the mono-nitrotoluene isomers before further nitration. Modified, or variants of TDI are also formed, which contain different functionalities and viscosity, for specific applications (Allport 2003).

Monomeric TDI is bi-functional, containing two isocyanate (NCO) functional groups attached to an aromatic parent compound (Streicher et al. 2000; Deschamps et al. 1998;

Nakashima 2002; Weyel and Schaffer 1985; Bello et al. 2007; Woolrich 1982; Bello et al. 2004). As a result of electrophilic aromatic substitution, TDI contains a net positive charge on each carbon atom of the functional group (Arnold 1957). Accordingly, polyols serve as nucleophilic agents, promoting addition of hydroxyl groups across the N=C bonds (Ulrich 1996). This reaction forms repeat units of NHCOO groups, which is a universal feature of all polyurethane products (Ashida 2007).

The chemistry of polyurethane manufacturing covers a variety of processes and isocyanate handling, including how the isocyanate is supplied and reacted (Allport 2003; Booth et al. 2009). Depending on the end-use product, processes may be open or closed systems, and TDI may be poured or sprayed. For example, continuous foaming lines of slabstock for making blocks of flexible foam are enclosed with either permanent or removable barriers (Cummings and Booth 2002).

With continuous throughput machines, flexible slabstock is produced at specialized plants using a variety of manufacturing processes (Ulrich 1996; Cummings and Booth 2002). The Maxfoam process is the most popular, which uses a fixed mixing head to deliver reactants into a trough. To achieve large blocks, high output machines are required, which need faster and longer conveyors. A foam line of a slabstock plant is delineated into six sections: (1) bottom and top paper/plastic feeders, (2) mixing head area, which includes control panels and area where the liquid foam mixture is blended and applied, (3) tunnel with bottom and side platen conveyors, (4) paper and plastic removal area, (5) cutoff saw, and (6) conveyor that feeds finished foam to a storage area for curing (Booth et al. 2009). “Buns” are formed after the slabstock foam is cut, which are approximately four feet by seven feet with a length of eight to 200 feet.

TDI has a relatively high vapor pressure compared to MDI (e.g., 0.0105 compared to 0.000005 mm Hg at 25 °C), so it is primarily an inhalation hazard (Allport 2003; Cummings and Booth 2002; Hygienists 2004). Airborne TDI species may be in the form of a vapor, as a mixture of vapor and aerosols, or as a component of a reacting mixture (Allport 2003). Accordingly, TDI is a pervasive hazard in all industrial, commercial, or manufacturing settings if present because of the high volatility (Agency 2011). Exposure can occur in all phases of its manufacture and use (Program 1986). Generally, reactive aerosols are generated through spraying mechanisms (Allport 2003). For example, surface finishing of furniture, which contains 50% 2, 4-TDI, is most commonly applied by spraying. Aerosol TDI concentrations have been reported as high as 200 $\mu g/m^3$ (Tsai et al. 2006).Overexposures have been reported in polyurethane foam production facilities (Ott, Diller, and Jolly 2003; Cummings and Booth 2002), as well as from consumer use of uncured TDI products (e.g., TDI-containing moisture cure urethane floor finish, spray applied sealants and coatings)(Agency 2011). Bystander exposures have also been reported from concrete patio sealants (Kelly, Myers, and Holdren 1999; Jarand et al. 2002), communities near toluene diisocyanate facilities (poster presentation), and polyurethane foam mattresses (poster presentation).

In general, exposure to diisocyanates is associated with adverse health effects of the respiratory tract, ranging from severe irritant effects on the mucous membrane causing chemical bronchitis, severe bronchospams, hypersensitivity, and pulmonary edema (Woolrich 1982; Hettick et al. 2012; Ruwona et al. 2010; Wisnewski, Hettick, and Siegel 2011; Hygienists 2004). Health effects from exposure are related to the physical state of airborne TDI, since particle size governs respiratory deposition (Streicher et al. 2000). TDI and other diisocyanates are globally recognized as the most common causes of occupational asthma with 5-30% of exposed workers

developing the disease, but mechanisms of disease pathogenesis remain poorly understood (Wisnewski and Jones 2010; Hettick et al. 2012; Ruwona et al. 2010; Wisnewski, Hettick, and Siegel 2011; Porter, Higgins, and Scheel 1975; Adams 1975; White et al. 1980). Based on the interplay of factors such as, route and duration of exposure, peak versus average exposure, chemical composition and physical form of isocyanate, and deposition site in the respiratory tract, partitioning value of isocyanate vapors, and host susceptibility—health outcomes remain equivocally defined (Meredith, Bugler, and Clark 2000; Ott, Diller, and Jolly 2003; Bello et al. 2004).

The Occupational Safety and Health Administration (OSHA) mandates a ceiling permissible exposure limit (PEL) for airborne, monomeric TDI of 140 micrograms per cubic meter ($\mu g/m^3$), which is equivalent to 20 parts per billion (ppb) (Administration). However, this PEL is based on an antiquated list of Threshold Limit Values (TLV) published by the American Conference of Governmental Industrial Hygienists (ACGIH), which prescribes exposure criteria for only monomeric TDI using a ceiling limit of 20 ppb (Bello et al. 2004; Administration). OSHA defines a ceiling value as an exposure limit that shall not be exceeded at any time during the working day for a particular substance (Administration).

OSHA does not mandate a time-weighted average (TWA) standard for TDI; however, the protocol for monitoring TDI is to assess the ceiling over a 15-minute period (Bello et al. 2004). Consequently, instantaneous concentrations may exceed the ceiling limit, while the average may very well be within the legal requirements of 20 ppb for airborne aromatic diisocyanates. This underestimation is an important caveat in regulatory compliance and sampling strategy for TDI since peak exposures may be more relevant than lower long-term, or cumulative exposures in

terms of risk for developing symptoms of asthma (Allport 2003; Mapp et al. 1988; Mapp et al. 2005; Chan-Yeung and Malo 1995).

In 2002, Cummings and Booth assessed and characterized airborne TDI exposures in workers at six flexible slabstock foam facilities in the United States (Cummings and Booth 2002). The slabstock lines operated between one and four hours, and the potential for exposure to either 2,4- or 2,6-TDI varied depending on the location of the worker monitored. Briefly, the work atmosphere may be enriched with 2,6-TDI since 2,4-TDI has a higher rate of reactivity (Booth et al. 2009; Allport 2003; Rando, Abdel-Kader, and Hammad 1984; Boeniger 1991).

Cummings and Booth collected a total of 403 airborne samples over 15-minute sampling periods (Cummings and Booth 2002). Four different categories of workers were monitored: Foam Head operator, Foam Line Operator, Saw Operator, and Bun Handler. While the Foam Head operator was least likely to be exposed to elevated levels of airborne TDI, the Foam Line Operator had the greatest overall potential. Like Foam Line Operators, Saw Operators had to enter the foam line tunnel, which increased their chances for an overexposure. The risk of exposure of a bun handler depended on proximity to a newly made, uncured bun. Twenty-four of these samples exceeded the 20 ppb ceiling-PEL. Overexposures to airborne TDI most likely occurred upon entering a foam line tunnel for maintenance, or close contact with freshly cut foam.

Accurate measurements of TDI in workplace atmospheres are crucial, especially for previously sensitized workers. Quantitative determinations rely on capturing a representative sample; however, isocyanates characteristically have a widely varying disposition in the workplace, especially in spray applications (Pronk et al. 2006; Redlich and Karol 2002; Rom and Markowitz 2007; Streicher et al. 2000; Allport 2003; Bello et al. 2004). Therefore, isocyanate

sampling and analysis is challenging (Streicher 1994; Streicher et al. 2000). Technical difficulty in quantitative determinations of TDI are due to episodic exposure profiles, high reactivity and adsorptivity to particulate matter, particle size and concomitant exposure to other isocyanate species, solvents, and other interfering additives (Allport 2003; Streicher et al. 2000; Redlich and Karol 2002; Lesage et al. 2007; Streicher 1994).

Currently, active sampling is typically achieved by use of an impinger filled with an absorbing solution or a reagent-coated air filter sampler. These two sampling systems contain a derivatizing agent to enrich analytical identification and quantitation of isocyanates based on the formation of a stable urea derivative with strong molar absorptivities (Levine 2002; Streicher et al. 2000). Other methods have been developed to separate the aerosol and vapor phases of TDI, which include dual and triple filter, denuder, and denuder –cascade impactor systems (Tsai et al. 2006; Nordqvist, Nilsson, and Colmsjo 2005; Nordqvist et al. 2005; Dahlin et al. 2008).

OSHA modified the original impinger method by substituting a 37-mm cassette containing a glass fiber filter (GFF) impregnated with 1-2PP for the impinger device (Allport 2003; Levine 2002). OSHA recommends drawing a known volume of air through an open-face cassette at 1 liter per minute (L/min) for a total volume of 15 liters (L). The OSHA Sampling Method 42 for TDI uses 1.0 milligram (mg) of the secondary amine to coat the GFF (Administration ; Administration). Following the completion of sampling, the OSHA recommends replacing the top cover and small plugs on the cassette, and sending to an accredited analytical laboratory. Upon receipt, the laboratory will remove the GFF from the cassette, placing it into a vial containing 2 ml of 90/10 ACN/DMSO desorbing solution.

The OSHA validated sampling and analytical method 42 using GFFs coated with 1,2-PP and an HPLC with a fluorescence detector (Administration). Experimental designs were tailored

to evaluate retention and extraction efficiency, detection limits, reliable quantitation limit, thermostability, and storability. Controlled atmospheres of TDI were not generated. Instead, a vapor spiking technique was used as an alternative to study the behavior of TDI once collected in an open-face cassette.

Working standards of 2,4 TDI-urea derivatives were prepared to avoid polymerization of the isocyanate during evaluations. Briefly, a solution of TDI and 1,2-PP in methylene chloride were mixed to form white slurry. Purified TDI derivative was obtained following precipitation, filtration, and hexane washing of the slurry. Stock solutions were prepared using DMSO. Subsequent dilutions of the stock were made using ACN to arrive at the working range of MDI. A conversion factor of 0.3479 was calculated by dividing the molecular weight of TDI by the molecular weight of the TDI-urea derivative. The amount of free TDI was then determined by multiplying the weight of TDI derivative by this conversion factor.

Vapor samples that were ten times the target concentration of TDI (140 $\mu g/m^3$) were generated to evaluate retention efficiency of a GFF. An average of 95% recovery of TDI was determined when 200 L of air with 12% relative humidity was drawn through the filter. However, when the relative humidity was increased to 78%, increasing amounts of TDI were lost with increasing volume of air (Table 4-1). Hydrolysis is a key player in determining the overall environmental and physiological (i.e., bioaccumulation) fate of airiborne TDI since hydrolysis from humidity has been shown to hydrolyze TDI to its corresponding amine. This scenario raises concerns regarding exposure to toluene diamine, a potential occupational carcinogen (Prevention 1989). Addtionally, concerns regarding persistence and stability of TDI under conditions of low humidity are also raised (Agency 2011).

TABLE 4-1. Percent recovery of TDI vapor spiked GFFs with increasing air volumes containing 78% humidity (Administration).

air volume, L	2,6-TDI (%)	2,4-TDI (%)
5.25	90.8	85.1
5.25	90.3	84.0
10.5	91.2	84.5
15.75	89.7	82.6
15.75	89.7	78.9
21.0	89.8	82.3
21.0	85.1	77.4
26.25	88.8	81.7
26.25	84.0	78.2
31.5	84.5	77.1
36.75	84.7	80.0
42.0	86.8	80.1
42.0	85.9	79.7
47.25	84.9	79.2
47.25	84.0	75.7
52.5	87.4	80.8
52.5	86.4	79.4

Isocyanate accessibility to the derivatizing agent is critical when using reagent-coated GFFs, especially in conditions with high humidity, spray applications using two-component systems, or both (Booth et al. 2009; Streicher 1994). Spray products rapidly cure with a half-life of less than two minutes. Micrographs of GFFs containing samples taken during spray applications show minimal contact with the reagent-coated fibers (Bell 1994). As a result, dispersal of the aerosol is negligible, and larger aerosols exhibit an inherent challenge of accessing the reagent (Streicher 1994). Consequently, isocyanates will be lost to competitive reactions within the aerosol mixture and underestimated. For example, Tsai et al. characterized airborne TDI concentrations in a furniture finishing workplace using five different samplers while examining the effect of sampling duration (Tsai et al. 2006). Aerosol concentrations were reportedly higher using a dual-filter system as compared to an annular denuder and triple filter

system. Additionally, only a nominal amount of TDI aerosol was quantitated after increasing sampling time from 15 to 30 minutes.

To improve the performance of filter methods, the filter may be removed from the cassette in the field and desorbed immediately after sampling in a vial containing a solvent miscible with the reactants (Karoly 1998; Streicher et al. 2000). When the filter is desorbed, the extracting solvent will dissolve both the derivatizing reagent and any un-reacted isocyanate, allowing the two to combine in solution and form a stable urea-derivative. Streicher et al. recommend desorbing samples in the field immediately after sampling whenever isocyanates are collected (Streicher et al. 2000). However, OSHA still prescribes filter desorption to occur at the analytical laboratory upon receipt of sample shipment.

Existing literature suggests that a significant difference exists between the results of field- and laboratory-desorbed methods; however, this difference has been identified only in field studies monitoring airborne MDI. A statistically significant difference was determined in side-by-side comparisons of FD and LD methods in two unrelated work environments (Karoly 1998; Schaeffer et al. 2013). Researchers consistently reported higher amounts of MDI were collected from FD samples. Karoly investigated FD and LD methodologies using a proprietary method, ICI Polyurethanes sampling and analytical Method I 1024G, revision 1.7, to determine airborne concentrations of MDI during application as a binder in four different wood mills manufacturing oriented strand board (Karoly 1998). This sampling method used 13-mm GFFs impregnated with 2mg of 1,2-MP and 2% diethyl phthalate. Immediate desorption was suggested to minimize the loss of MDI otherwise blocked from the derivatizing agent by a layer of dust. MDI coated on wood dust eventually reacted with itself, moisture, or the dust.

Schaeffer et al. (Schaeffer et al. 2013) assessed the suitability of FD and LD methods in the spray-on truck-bed liner industry, which generates aerosols containing a mixture of MDI and polyol. In this study, the OSHA sampling method 47 was used, which prescribed 37-mm GFFs impregnated with 1,2-PP. The FD samples yielded consistently higher MDI concentrations than the LD samples, which suggested that immediate desorption minimized isocyanate loss and potential underestimations. Higher amounts of MDI associated with the FD methodology were attributed to dissolution of the analyte and derivatizing; MDI and reagent were able to make contact despite the presence of competitive reactants. Spray droplets and large particles have been conjectured as a limitation of the reagent coated GFFs due to minimal contact between the isocyanate and the coated fibers. Since STBL application generates aerosols with a wide range of particle sizes containing a mixture of MDI and polyol, these results were not unexpected.

Kuck et al. investigated partial rate factors between monomeric diisocyanates and six different derivatizing reagents in test solutions (Kuck 1999). Reaction rates were shown to vary by orders of magnitude between diisocyantes. Inherent properties such as: selectivity, stability, sensitivity, and geometry of both the agent and diisocyanate likely accounted for such reactivity differences (Kuck 1999). Rapid diisocyanate derivative stabilization is a key step in the prevention of isocyanate loss due to side reactions with other compounds or artifacts. Consequently, reaction rates have the potential to significantly impact the accuracy of analytical determinations of airbore diisocyanate monitoring (Kuck 1999).

Tremblay et al. evaluated competitive rates of four different derivatizing agents with aliphatic and aromatic diisocyanates (Tremblay et al. 2003). Relative reactivity was demonstrated to be a function of the chemical structure of the diisocyanate, even between TDI isomers (e.g. 2,4- and 2,6-TDI). Using specific software, physicochemical properties (including

electron density and electrophilicity) of both TDI functional groups were found to be analogous, attributing relative reactivity to the three-dimensional difference of each TDI isomer. Since the 2, 6-TDI isomer is an asymmetrical molecule; the methyl group substituted at the one position on the benzene ring causes equal hindrance on the isocyanate groups. Consequently, 2,4-TDI is much less hindered, and yields a greater derivatization percentage. Structural differences were additionally noted in the Tremblay et al. investigation between MDI and TDI with secondary amines. Such differences in reactivity were examined by the formation of the urea derivative and the corresponding ultraviolet UV response. Varying response factors and retention times of MDI and TDI were easily observed when individual chromatograms were overlaid on each other.

Relevant competitive rate studies have emphasized a disparity in reactivity between MDI and TDI with a gamut of secondary amine derivatizing agents (Kuck 1999; Streicher et al. 1996; Wu et al. 1987). However, anecdotal variations still exist between MDI and TDI in relation to 1,2-PP, as this secondary amine was not evaluated. Therefore, it is very important that additional investigations are conducted that accurately assess the potential for disparity between MDI and TDI reactivity with 1,2-PP, specifically analyzed by both field and laboratory desorbed sampling methods.

While variants of sampling methods for TDI exist for particular workplace environments (Streicher et al. 2000), companies have greater incentive to use the OSHA methods in order to comply with current regulation. However, aerosols of various particle sizes were not included in the evaluation of measurement accuracy. Since aerosols are a key constituent of a typical exposure scenario involving spray applications of isocyanates, more research is needed to narrow the gap in understanding the effects of particle size on quantitative determinations of MDI.

The purpose of this research was to determine the accuracy of FD and laboratory LD methods using aerosolized TDI (underivatized) instead of a liquid spike of urea-bound MDI. Results were expected to reflect the affect of curing and hydrolysis of free isoycanate functional groups. To accomplish this goal, an isocyanate aerosol generating system (IAGS) was designed and tested to provide an evaluation consistent with a spray isocyanate exposure scenario. This is the first attempt, to the author's knowledge, to evaluate these methods using atomized TDI instead of a vapor spike.

Consistent with other investigations, analytical results from FD filters was anticipated to closely approximate theoretical amounts of TDI while LD filters would significantly underestimate TDI. This underestimation is attributed to the inherent challenges of stabilizing the chemical in LD filters. Furthermore, with the absence of other compounds present in technical grade TDI, pure grade TDI was conjectured to react faster with the derivatizing agent, 1,2-PP, yielding higher amounts of TDI.

MATERIAL AND METHODS

Chemicals and Equipment

Pure TDI (CAS No. 584-84-9) was purchased from Sigma-Aldrich (St. Louis, MO) containing 95% 2,4-TDI and 4% 2,6-TDI. BDH® American Chemistry Society (ACS) grade toluene (CAS No. 108-88-3), suitable for histology and cytology application, was acquired from the Colorado State University Environmental Health Services' chemical redistribution program (unopened) (Fort Collins, CO).

A KDS 200 Two-Syringe Infusion Pump was purchased from KD Scientific (New Hope, Pa.). An EZ-STARTER airbrush set with an atomizing nozzle was purchased from PAASCHE®

Airbrush Company (Chicago, IL), which included air hose with couplings that connected to a ¼ inch Victor® CGA 346 two-stage gas regulator (Denton, Texas) fitting assembled to a size 300 grade D high pressure breathing air purchased from Airgas (Fort Collins, Colorado).

The Wisconsin Occupational Health Laboratory (WOHL) provided 37 mm, 3-piece cassettes containing glass fiber filters (GFF) treated with one milligram (mg) of 1, 2- pyridyl piperazine along with desorption vials containing two milliliters (ml) 90% acetonitrile and 10% dimethyl sulfoxide (90/10 ACN/DMSO).

Description of Isocyanate Delivery System Design

A small-scale spray system (Figure 4-1) was designed and built to load GFFs with isocyanate test aerosols to evaluate percent recovery (see Chapter 2). Validation of IAGS design was based on experimental values using water and preliminary MDI samples (see Chapter 2). While differences in rates of derivatization are expected between MDI and TDI, the same accuracy and precision of the IAGS from these validation studies are assumed to apply to the delivery of TDI.

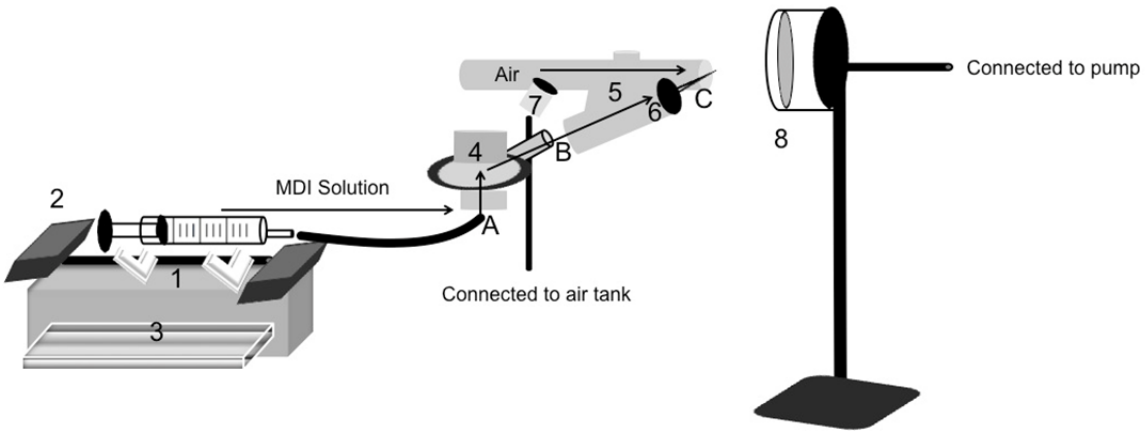

FIGURE 4-1. TDI Delivery System and Sample Collection: 1, the barrel of the syringe rests in the "V" and a retaining arm (not shown) secures the syringe in place; 2, pusher block mechanism that travels along guide rods when drive nut is engaged and depresses syringe plunger at set rate; 3, keypad used to navigate displayed menu features and selection of functions (e.g., syringe manufacturer, size, and diameter, units and rate settings, and directional mode); 4, airbrush adaptor; 5, airbrush; 6, adjustable nozzle; 7, compressed air adaptor; 8, 37-mm open-face cassette; A, connection to the airbrush adaptor; B, connection of adaptor to the airbrush; C, exit out of the nozzle.

The reported accuracy and reproducibility of the KD Scientific 200 Two-Syringe Infusion Pump were ±<1% and ±<0.1%, respectively; however, quality assurance was independently conducted at specific points within the system (Items A, B, C, Figure 4-1) (see Chapter 2). Additionally, a proof of concept was performed using an MDI working solution corresponding to 1.3µg ml^{-1} of toluene dispensed at a flow rate of 0.193 ml $min.^{-1}$ (see Chapter 2).

The study design of this investigation will use the single-flow operation (see Chapter 2) of the KD Scientific 200 Two-Syringe Infusion Pump to deliver known amounts of TDI dissolved in toluene. Accordingly, percent recovery between atomized TDI and the derivatizing agent, 1-2PP will be evaluated using the GFF FD and LD methods.

Toluene is anticipated not to interfere with the derivatization between TDI and 1,2-PP. The NIOSH Method 5521, an impinger method, uses a solution of 1,2-PP in toluene to capture

monomeric MDI and TDI. Impinger methods have been shown to enhance derivatization kinetics of large MDI/polyol aerosols in the field (Lesage et al. 2007). Additionally, Dahlin et al. prepared toluene solutions containing 2,4-TDI to investigate the suitability of a denuder-impactor system for the separation and analysis of TDI vapor and aerosols in different particle size fractions (Dahlin et al. 2008). Furthermore, Dahlin et al. prepared TDI-derivative solutions using di-*n*-butylamine, a secondary amine, in toluene to serve as HPLC standards.

In Chapter 2, toluene was determined not to interfere with the reaction between airborne MDI (technical and pure-grades) and 1, 2-PP using a series of dilutions with an increasing volume of toluene (see Chapter 2). Each dilution was a geometric progression of MDI concentrations with a common ratio of 2. Aerosolized samples were collected using the GFF FD sampling methodology and sent to the Wisconsin Occupational Health Laboratory (WOHL) for HPLC quantitation. A trend of decreasing MDI with respect to the dilution factor was detected in each set of MDI samples (i.e., pure and technical grade). The common ratio of technical grade MDI was in agreement with that of pure grade MDI.

Using a Grimm Dust Monitor Model 1.108 (Grimm Technologies, Inc., Douglasville, GA), the IAGS particle size distribution was determined. Number concentrations reported by the Grimm were converted to a mass concentration using the following equations:

(1)

$$\text{Density}_{\text{mixture}} = 1/(\%_{\text{mdi}} / \text{density}_{\text{mdi}}) + (\%_{\text{toluene}} / \text{density}_{\text{toluene}})$$

(2)

$$dM_i = \pi/6\ (\rho_p D^3_{p,i})\ dN_i$$ (Peters, Ott, and O'Shaughnessy 2006)

where ρ_p represented particle density, and $D^3_{p,i}$ and dN_i represent the midpoint aerodynamic diameter and number concentration measured in the *i*-th channel of the Grimm (Peters, Ott, and O'Shaughnessy 2006).

Equation 1 was used to calculate the density of each MDI working solution, and equation 2 was used to calculate the particle mass concentrations at each size distribution channel of the Grimm (see Chapter 2). Approximately 95% of the aerosol mass concentration was associated with particles greater than 2 μm while 95% of the aerosol number concentration was associated with particles less 2 μm. The majority of the number concentration (75%) was contained between 0.35 and 0.725 μm. Particles greater than 3.5 μm contained 75% of the mass during the process

The fundamental assumptions of this research are:

- The IAGS will deliver atomized TDI consistent with the reported accuracy and reproducibility of the KD Scientific 200 Two-Syringe Infusion Pump
- The solvent toluene is miscible with TDI making a homogenous solution of working standards
- The solvent toluene does not interact with the derivatizating agent, 1,2-PP
- A wide range of TDI particle sizes will remain on the filter
- By comparing observed to theoretical, accuracy and consistency of each diisocyanate field sampling method can be demonstrated.

Preparation of TDI Solutions

Pure grade TDI*:*

The weight-to-weight ratio of monomeric TDI (2,4-TDI) was reported as 0.95g/g of sample. To prepare pure TDI standards, a 1mg/ml stock solution was prepared. Approximately, 100 mg of pure grade TDI was weighed out on a Denver Instrument M-series 220D analytical balance and mixed with 10mL of toluene (equation 1). This solvent is readily miscible with isocyanates, and conjectured not to interfere with derivatization of the isocyanate..

(1)

1mg/mL x 100mL = 100mg of TDI;

From the 1mg/ml TDI stock solution, working toluene solutions containing monomeric TDI concentrations corresponding to an approximate log mass concentration of micrograms (μg) ml^{-1} (Table 4-2) were made using equation 2 (and confirmed by HPLC at the WOHL): The mass concentration in each of these TDI working solutions was comparable with the MDI working solutions used in Chapter 3.

(2) $Concentration_1 \times Volume_1 = Concentration_2 \times Volume_2$

TABLE 4-2. Technical grade TDI solutions prepared using equation 2.

Category	MDI concentration
Working solution 1	0.94 μg ml^{-1}
Working solution 2	2.1 μg ml^{-1}
Working solution 3	4.7 μg ml^{-1}
Working solution 4	10 μg ml^{-1}

Since the IAGS was validated at 0.193 and 0.380 ml $min.^{-1}$, working solution 1 was metered at both dispense flow rates to maintain an approximate logarithmic scale. Subsequently,

workings solutions 3-5 were metered at 0.380 ml $min.^{-1}$. As a consequence, amounts of TDI delivered were approximately 20% and 40% of the original amount based on these adjusted flow rates.

Application of these TDI working solutions required increasing the sampling rate of the Mines Safety Appliances (MSA) Electronic Laminar Flow (ELF) pump while decreasing air volume to achieve practical concentrations of airborne MDI. Briefly, the recommended air volume and sampling rate in OSHA method 47 was 15 L and 1 L/min. To approach the OSHA ceiling PEL, only 2 L of air were collected at 2 L/min. For example, if working solution 1 was delivered at a flow rate of 0.193 mL $minute^{-1}$ for 1 min., theoretically 181.4ng (equation 3) was delivered to a GFF, which is the approximately 12 ppb (equation 4).

(3) TDI solution ($\mu g\ ml^{-1}$) x dispensed flow rate ($ml\ min^{-1}$) x sample time (minute) = μg

(4) $mg\ m^{3\ -1}$ = ppm (MW)/24.45 (P_o/P_m) (T_m/T_o)

	$mg\ m^{-3}$ =	Mass concentration based on HPLC results and volume of air collected
	ppm=	parts per million
	MW=	Molecular weight of MDI
where :	P_o=	Normal pressure
	P_m=	Measured pressure
	T_o=	Normal temperature
	T_m=	Measured temperature

Study Design

Accuracy of FD and LD methods was evaluated using underivatized monomeric TDI in the form of an aerosol-spiking technique (i.e., spray loading test filters). Methods were assessed on their capability to collect, derivatize, and quantify ultra-trace amounts (nanograms) of TDI. A logarithmic scale of TDI concentrations was used to determine if accuracy was modified or influenced by an interaction with concentration. Briefly, spray loading of TDI on a filter will produce discreet droplets in the immediate vicinity of limited 1,2-PP; however, vapor spiking

will yield gas molecules, which are less than 1 nanometer in diameter. Particles less than 2 µm are optimally sampled by GFF methods since impinge flasks have a low collection efficiency of particles in the sub-micron range (Dahlin et al. 2008).

Collection of Atomized Monomeric TDI

Atomized TDI was collected from five different concentrations using an open-face sampling technique described in OSHA Method 47; however, flow rate of the sampling pump connected to the cassette was operated at 2 liters per minute (L/min) instead of 1 L/min. With this adjusted flow rate, practical airborne concentrations in parts per billion were achieved within a short sample time of one minute. An MSA ELF pump was calibrated at a flow rate of 2 L/min using a Dry Cal DC Lite-DCLT 5K rev 1.08 (Pompton Plains). One sample was collected at a time, and the flow rate was checked post-sampling to ensure they were within ±5% of the original flow rate. The MDI sampling cassettes and desorbing solution were stored in a refrigerator before use as prescribed by OSHA method 47 and WOHL method LC48.

A net increase in filter weight, as a result from aerosolized water, was not included in the validation of the IAGS; however, filters were still weighed using a Denver Instrument M-series 220D analytical balance (Arvada, CO) before and after collection of aerosolized isocyanates to ensure comparable deliveries (see Chapter 2).

After the sampling cassettes were removed from the apparatus and weighed, the top cover and small plugs of the LD cassette were replaced. The FD filter was removed using forceps and placed into a glass vial containing 2 mL of 90/10 ACN/DMSO desorbing solution. The researcher agitated the vial gently to ensure that the entire filter was saturated with desorbing solution. The vial was sealed with the cap provided by WOHL and wrapped with parafilm around the top of the vial to prevent leaking.

Accuracy of the FD and LD techniques was evaluated based on comparing HPLC-quantitated results from the WOHL, an American Industrial Hygiene Association (AIHA) accredited laboratory, to theoretical results. Based on the IAGS dispensed flow rate of a TDI working solution and sample time, a theoretical result was calculated (Table 4-3). By comparing observed to theoretical, accuracy and consistency was demonstrated for each diisocyanate field sampling method.

TABLE 4-3. Theoretical amounts of pure grade TDI delivered by the IAGS based on concentration, flow rate, and time

Category	Concentration (μg/ml)	IAGS Flow Rate (ml/min)	Sample Time (min.)	Theoretical Amount of MDI (ng)
Working solution 1	0.94	0.193	1	181
Working solution 2	0.94	0.380	1	357
Working solution 3	2.1	0.380	1	798
Working solution 4	4.7	0.380	1	1786
Working solution 5	10.0	0.380	1	3800

Spray loading FD and LD filters using pure grade MDI were collected on two different days. FD and LD pure grade MDI samples were collected on the same day in May, 2012. In one day, working solutions 1-3 were applied to FD and LD samples while solutions 4 and 5 were collected the following day.

Statistical Analysis

Statistical analyses were performed using the Statistical Analysis System (SAS) computer program (version 9.2, SAS Institute Inc., Cary, NC). A sample size calculation and power analysis were not performed *a priori*. Briefly, variance and effect size of loading GFFs with underivatized TDI in a controlled setting were unknown. Such MDI studies were conducted; however, since different derivatization rates of MDI and TDI were expected, the variability and effect size between FD and LD results observed in the MDI study were not suitable for this study. Therefore, only post-hoc analyses were conducted. Retrospectively, effect size was determined to evaluate practical relevance of statistically significant differences. P-values were used to assess accuracy of each method. Values of $p<0.05$ were considered significant.

Accuracy of desorption method was defined as 100% recovery of TDI compared to theoretical amounts; calculated from concentration and dispensed flow rate of the syringe pump. Briefly, comparing experimental results to theoretical produced a percent recovery for each FD

and LD replicate. A scatterplot of studentized residuals versus predicted values was performed to assess normality and independence.

The residuals were approximately normal and independent following log-transformation of the data. Accordingly, a log-based ratio of zero represented 100% recovery based on taking the logarithm of 1, denoting an experimental amount equal to the theoretical value. Using the log-based ratio as the dependent variable in the statistical model, FD and LD differences from zero were analyzed using least square means to determine if a statistically significant difference existed at each concentration for a fixed loading.

General Linear Models were used to assess the multivariable relationship between loading mechanism, desorption method, and concentration. Least square means of the log-based ratio and standard error were used to compare the mass (ng) of MDI collected by FD and LD methods at each loading concentration. To investigate interactions between each variable (i.e., loading mechanism, desorption method, and loading concentration), a three-way analysis of variance (ANOVA) was performed with four degrees of freedom. Power was increased using a three-way parametric ANOVA since the loading mechanism and desorption method were categorical variables.

RESULTS AND DISCUSSION

A total of 50 TDI samples were collected using FD and LD methods. Quantitative determinations of TDI from spray loading were reported in units of mass (ng/sample). Descriptive statistics of FD and LD TDI data collected at each concentration are presented as the mean ± standard deviation and range (Tables 4-4 and 4-5).

TABLE 4-4. Summary of analytical results from spray loading test filters with pure grade TDI.

Grade of MDI	Working Solution	Desorption Method	Mean Mass (ng) (± SD; range)	Theoretical Mass (ng)
Pure	1	Field	170 (±12; 160-190)	181
Pure	2	Field	290 (±16; 270-310)	357
Pure	3	Field	592 (±28; 570-620)	798
Pure	4	Field	1300 (±130; 1200-1500)	1786
Pure	5	Field	2980 (±268; 2700-3400)	3800
Pure	1	Laboratory	148 (±4; 140-150)	181
Pure	2	Laboratory	282 (±13; 260-290)	357
Pure	3	Laboratory	590 (±19; 570-620)	798
Pure	4	Laboratory	1340 (±152; 1200-1600)	1786
Pure	5	Laboratory	3040 (±261; 2700-3300)	3800

TABLE 4-5. Summary of airborne concentrations from spray loading test filters with pure grade TDI.

Grade of MDI	Loading Mechanism	Desorption Method	Airborne Concentration (parts per billion) (± SD; range)
Pure	Spray	Field	12.8 (±0.84; 12-14)
Pure	Spray	Field	18.7 (±2.3; 15-22)
Pure	Spray	Field	39.6 (±4.8; 32-45)
Pure	Spray	Field	82 (±3.6; 77-86)
Pure	Spray	Field	146 (±11.4; 130-160)
Pure	Spray	Laboratory	10.3 (±0.73; 9.3-11)
Pure	Spray	Laboratory	19.5 (±1.4; 17-21)
Pure	Spray	Laboratory	37.6 (±1.7; 35-39)
Pure	Spray	Laboratory	79 (±3.7; 74-82)
Pure	Spray	Laboratory	138 (±8.4; 130-150)

Percent Recovery of Technical Grade TDI

In this analysis, the mean FD and LD data were compared to theoretical amounts of pure grade TDI to obtain a percent recovery, or a ratio of observed values to theoretical values. A ratio of one represented a 100% recovery of MDI. A percent recovery, least squares mean

(LSMEAN), and attendant p-value is shown for each loading mechanism, desorption method, and working solution of MDI in Table 4-6.

TABLE 4-6. LSMEANs associated with spray loading pure grade TDI

Loading	Desorption	Working solution	Log 10_ratio LSMEAN	Pr> |t|	Percent recovery
Spray	Field	1	-0.02810029	0.0487	94.0%
Spray	Field	2	-0.09078767	<0.0001	81.2%
Spray	Field	3	-0.13006324	<0.0001	74.1%
Spray	Field	4	-0.13297643	<0.0001	74.0%
Spray	Field	5	-0.10693495	<0.0001	78.4%
Spray	Laboratory	1	-0.08757996	<0.0001	81.2%
Spray	Laboratory	2	-0.10280314	<0.0001	79.0%
Spray	Laboratory	3	-0.13132318	<0.0001	74.0%
Spray	Laboratory	4	-0.12685520	<0.0001	75.0%
Spray	Laboratory	5	-0.09820142	<0.0001	80.0%

Spray Pure Grade TDI

The least squares means (LSMEAN) were calculated (Table 4-5) using the general linear model (GLM) to integrate the effects of desorption method, loading mechanism, and loading concentration.

Data were log-transformed to make inferences using a ratio of observed over theoretical. Normality and independence of data were observed in a studentized residual versus predicted plot, illustrated in Figure 4-2. No outliers were observed in this data set that corresponded to a delivery or measurement error.

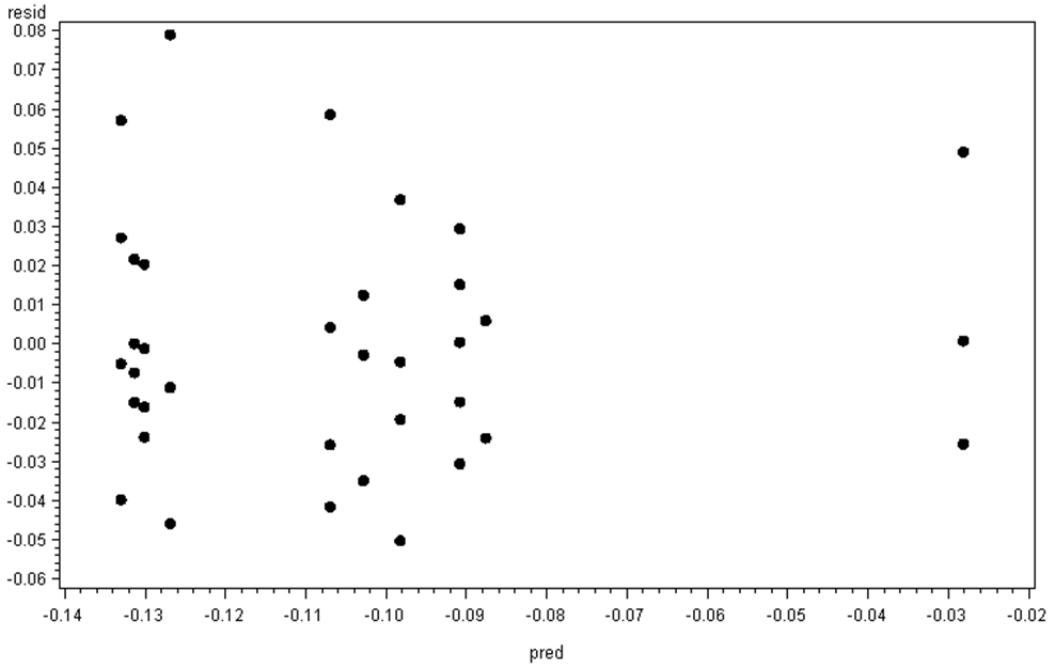

FIGURE 4-2. Studentized residual versus predicted plot of pure grade TDI data.

Using the general linear model, relationships between analytical results and individual variables were analyzed. Seven independent variables were included in this model (i.e., two desorption methods, and five working solutions of TDI). A p-value of less than 0.0001 was demonstrated in the omnibus test of this model (i.e., testing the null hypothesis (H_o) that none of the variables linearly predict the percent recovery of TDI). Using an alpha of 0.05, this p-value was considered significant, and the null hypothesis was rejected on this basis. Accordingly, the alternate hypothesis (H_a) that at least one of the independent variables does linearly predict the dependent variable of percent recovery was accepted.

Data were balanced in this model indicating that the factors in this model were orthogonal. Accordingly, type I and III sums of squares and F-values, which were calculated to determine the presence of interactions, were equal. The sum of squares (SS) from both tests added up to yield the sum of squares for the model.

Briefly, Type I, also called sequential tests, incrementally added factor effects to the model, considering one at a time in the order they were entered. Accordingly, the hypothesis depended on the order. Comparatively, Type III SS tests consider the effect of the independent variable as if it were entered last in the model (i.e., the independent variable is tested among all other variables included in the model).

While a significant F-value of 5.26 (p-value <0.0001) was shown in the omnibus test, the main effect of desorption alone was not found to predict percent recovery with an F-value of 1.26 (Table 4-7). The F-value associated with concentration was 9.34, which produced a p-value < 0.0001. But concentration was linearly related to percent recovery after accounting for desorption. Significant two-way interactions between desorption and concentration was not observed. As shown in Figure 4-3, FD and LD LSMEANs approximately parallel each other. The concentration of working solution 1 did show divergence in LD LSMEANs as compared to FD LSMEANs with a difference between means of 0.059480; however, the difference between all other LSMEANs was not large enough at any of the other concentrations to show an interaction (Table 4-8).

TABLE 4-7. Sum of square results, including F-, and p-values, from the GLM.

Source	**Degrees of Freedom**	**Type I and III Sum of Squares (F-value; p-value)**
Model	9	0.04517274 (5.26; <0.0001)
Desorption	1	0.00167622 (1.76; 0.1927)
Concentration	4	0.03567890 (9.34; <0.0001)
Desorption*Concentration	4	0.0078761 (2.05; 0.1061)

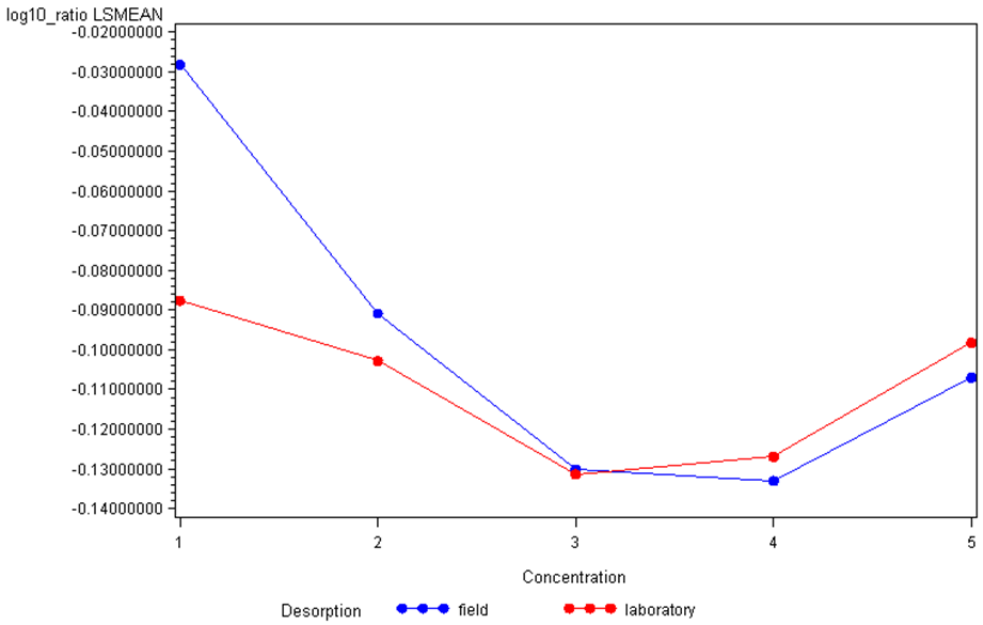

FIGURE 4-3. A plot of log_{10} ratio LSMEANs versus concentration of pure grade TDI working solutions.

TABLE 4-8. Difference between FD and LD means with associated p-values

Desorption method		Technical TDI working solution	Difference between LSMEAN s	Pr> | t |
Field	Laboratory	1	0.059480	0.0041
Field	Laboratory	2	0.012015	0.5422
Field	Laboratory	3	0.001260	0.9489
Field	Laboratory	4	-0.006121	0.7557
Field	Laboratory	5	-0.008734	0.6574

Generally, analytical results from each loading concentration of TDI did not vary with desorption method. Differences between FD and LD sampling methods were not significant following sample collection of atomized working solutions 2-5. However, a significant difference was observed between FD and LD percent recovery of working solution 1. A difference of 0.059480 with a p-value of 0.0041 was reported between FD and LD LSMEANs in the GLM output. As shown in Table 4-7, FD LSMEANs associated with working solutions 1-3 were greater than LD LSMEANs, which accounted for the positive differences observed. As shown in Figure 4-3, LD methods exhibited higher estimates than results obtained from

desorption of filters in the field treated with working solutions 4 and 5. Accordingly, these differences were negative.

A higher net increase in filter mass did not account for the significant difference between FD and LD methods observed at working solution 1. Briefly, net filter weights were recorded from pre- and post-sampling weights (Table 4-8). This measure was an attempt at quality control for comparability of FD and LD filters. While the variability between-test weights in this study were less as compared to the MDI study, a comparison between filter weights did not prove to be a useful indicator of bias due to the evaporation of toluene. As noted in Chapter 3, higher filter weights were not unequivocally associated with higher amounts of isocyanate. For example, FD filters that were sprayed with working solution 1 had a net median mass of 46 mg after collection. These FD filters demonstrated a 94% recovery of atomized TDI. On the contrary, results from the gravimetric analysis of the corresponding LD filters showed a 6 mg increase in net median mass after collection of atomized TDI (Table 4-8). Despite the increase in net filter mass, the average amount of TDI recovered from LD filters loaded with working solution 1 was 22 ng less than the FD average amount. This decrease in the amount of TDI coincided with only an 82% recovery, which was significantly less than that reported for FD filters. Additionally, a higher net increase in the average mass of FD filters was observed after treatment with working solutions 4 and 5 (Table 4-9); however, LD filters yielded a slightly higher percent recovery. For example, a difference of 12 mg was detected after filters were treated with working solution 5, but LD filters showed a percent recovery of 80% compared to 78.4% of FD filters. Putatively, toluene did not uniformly evaporate. Filters were considered comparable based on the high performance of the IAGS observed during validation.

TABLE 4-9. Net increase in FD and LD filter mass treated with pure grade TDI working solutions 1-5

Loading	Working solution	FD net increase in filter mass (± SD)	LD net increase in filter mass (± SD)
Spray	1	46 mg (± 11mg)	52 mg (± 4mg)
Spray	2	104 mg (± 5mg)	102 mg (± 13mg)
Spray	3	100 mg (± 12mg)	104 mg (± 9mg)
Spray	4	112 mg (±11mg)	104 mg (±17mg)
Spray	5	102 mg (± 16mg)	90.6 mg (±11mg)

Collection of discreet droplets of pure grade TDI used with IAGS significantly underestimated expected amounts of TDI. FD and LD filters treated with atomized TDI demonstrated a significant underestimation of recovered TDI compared to the theoretical amount across all working solutions (Table 4-5). An underestimation of TDI from LD methods was not unexpected; however, FD methods were conjectured to closely reflect theoretical amounts of TDI. Additionally, a significant difference between desorption methods was also anticipated based on MDI field studies that included side-by-side comparisons of FD and LD filters (Karoly 1998; Schaeffer et al. 2013). Briefly, FD methods consistently yielded higher amounts of MDI in two unrelated work environments. Immediate desorption was concluded to minimize loss of isocyanate either blocked from the derivatizing agent (e.g., a layer of wood dust), or from irregular or minimal surface contact of a large aerosol with the GFF (e.g., spray applications). Since a range of aerosol particle sizes of TDI was anticipated, immediate desorption was expected to render the isocyanate to a stable urea for quantitative determinations.

Characterization of the size distribution of TDI aerosols was not conducted in this study due to concerns of compatibility with the GRIMM Dust Monitor Model 1.108. While this instrument was used in Chapter 2 to determine particle sizes of MDI aerosols, TDI aerosol may exist in gas and particle size fractions based on the vapor pressure of TDI. Using the Grimm to interpret the results would have been misleading based on the difficulties and challenges

observed in the particle measurements of MDI aerosols. For example, the MMD was anticipated to increase as the concentration of each working solution of MDI increased; however, MDI working solutions 3-5 showed a decrease. This observation was most likely a result of an increase in the density of the particle, in combination with evaporation of toluene. Since more MDI was present, smaller particles containing more MDI were generated, shifting the distribution toward smaller particle sizes. Additionally, the effect of evaporation of toluene was more noticeable in the higher concentration working solutions since less toluene was present. Future work is needed to determine the size distribution of TDI aerosols emitted from the IAGS. Using instrumentation such as the aerosol particle sizer (APS), TSI APS 3321 (TSI Incorporated, Shoreview, MN, USA) in combination with a condensation particle counter connected to a Scanning Particle Sizer (TSI Inc., St. Paul, MN, USA) would provide a suitable particle diameter range.

If a similar trend of particle size distribution from the IAGS was assumed for TDI aerosols as MDI aerosols, then the difference observed between FD and LD methods related to collection of aerosolized TDI working solution 1 may be attributed to particle size. Under this assumption, atomization of working solution 1 produced large aerosols composed of mostly toluene with a small amount of dissolved TDI. Evaporation of toluene in these lower TDI concentrations was expected to take longer than the working solutions with higher concentrations. Accordingly, these larger aerosols may have preserved the TDI over the 1-minute sampling time since toluene was in such excess, insulating TDI from competitive reactants. Consequently, immediate desorption influenced these results based on the assumption that large aerosols were present. Immediate desorption promoted derivatization of the isocyanate through

dissolution of the reactants, which dispersed the aerosol, interfered with curing reaction, and brought TDI and 1,2-PP together (Streicher et al. 2000).

While FD results yielded a 94% recovery of atomized TDI, these results were still significantly lower than the theoretical amount. As a result, loss of TDI occurred. This loss was attributed to volatilization, or competitive reactions with humidity, or both. TDI is a semi-volatile organic compound (Tsai et al. 2006) with a vapor pressure of 0.01 mmHg at 25°C. Therefore, TDI may partition between the gas and particle phases (Melin et al. 2001). Previous studies have shown that the predominant fraction of TDI is in the gas phase during thermal degradation of flexible polyurethane foam, discharge of all flexible polyurethane foam materials into a foaming tank, and during spraying applications (Tsai et al. 2006; Melin et al. 2001; Karlsson et al. 2000). Therefore, volatilization of TDI may have occurred once airborne since TDI vapors at room temperature have been reported (Industry). Losses to the inner wall may have occurred since the IAGS nozzle was flush with the inside surface of the open cassette. Mao et al. reported that a modified closed-face cassette was more suitable than the open-face sampler for collection of airborne TDI (Mao, Chen, and Lin 2000). The modified closed-face sampler contained 1,2-PP coated filters on the inside of the cover pieces, as well as along the whole inner wall of the two-piece cassette. Distribution of aspirated TDI within the modified closed-face cassettes showed that almost 30% of TDI adsorbed on the top and middle rim of the cassette. However, these results were based on test atmospheres since the distribution of collected TDI mass may be affected by the fraction of TDI in vapor or particle form. A comparison of these samplers in a flexible polyurethane foam plant demonstrated that the open-face cassette collected 21% less 2,4-TDI than modified closed-face cassette.

Adsorption of TDI to the inner wall of the open-face cassette may account for the loss observed in the LD results reported for working solution 1. This loss of TDI was marked by a 13% difference in recovery compared to FD methods. Volatilization of TDI would have occurred after contact with the filter since FD recovered a higher amount of TDI. Since smaller particle sizes of TDI aerosols, which would have enhanced derivatization kinetics, were assumed in working solution 1 after toluene evaporation, volatilization of TDI either occurred simultaneously with evaporation of toluene (after a certain amount of time), or volatilization occurred from the top layer of agglomerated isocyanates on the filter. Volatilization would not have occurred after contact with 1,2-PP since the TDI would have been stabilized by formation of a urea compound.

Competitive reactions with water vapor at low relative humidity may also account for the loss of TDI in LD filters, as well as FD filters, spray loaded with working solutions 1-5. Tsai et al. measured airborne TDI concentrations using five different samplers to evaluate the effect of sampling time and humidity on aerosol and gaseous TDI concentrations (Tsai et al. 2006). These investigators found that TDI concentrations decreased as a result of low and high relative humidity since the water vapor reacted with collected TDI molecules.

A 13% decrease in percent recovery of FD results was observed between working solutions 1 and 2, which marked the only decrease in this data set. The percent recoveries were steady in working solutions 2-5, showing no significant differences between FD and LD results. This stabilization indicated that loss of TDI was occurring at an equally rapid rate between these two methods, even though FD methods were anticipated to capture TDI more effectively.

A similar profile of atomized TDI percent recovery was presented in pure grade MDI (see Chapter 3). Loss of isocyanate to competitive reactions with water vapor most likely accounts for

the low percent recovery observed in both this study and the MDI study. While volatilization of TDI may exclusively account for the loss of isocyanate (i.e., to the inside walls of the cassette), MDI has a much lower vapor pressure than TDI, and was not anticipated to volatilize during that study. As water vapor from ambient air was drawn onto the filter by the sampling pump, either hydrolysis of the isocyanate to its respective diamine occurred, or a polymeric urea was formed. For example, MDI most likely forms plaques of polyurea following contact with water (Allport 2003).

This is the first study to atomize underivatized TDI in a controlled setting to investigate percent recovery. TDI was applied to filters using the IAGS. Based on the similar profile of percent recovery as pure grade MDI, a relative difference in derivatization kinetics between TDI and MDI was not observed. This finding was in agreement with the Tremblay et al. investigation of relative differences in reactivity of four diisocyanates, including MDI and TDI, with four derivatization agents (Tremblay et al. 2003). A greater difference in reactivity was observed when a hindered aromatic diisocyanate (i.e., MDI or TDI) was reacted in acetonitrile or acetonitrile doped with water. When competitive reactions occurred in the reaction solvent, toluene, intermediate differences were produced as compared to the other two solvents. While toluene was not used as a reaction solvent in this study, it did not negatively influence derivatization or further reduce the percent recovery.

FD and LD percent recovery results were most likely related to the physical form of TDI, which was not modified by desorption method. Collection of TDI droplets limited the access of TDI to the derivatizing agent while vapor spiking a solution of TDI promoted diffusion that was carefully directed by a Leur tip inserted into the inlet of a closed cassette. The OSHA reported that humidity affects the ability of a glass fiber filter to retain derivatized TDI (Administration).

In two different replicates in the OSHA experiment, an air volume of approximately 15 and 20 liters of humid air (78%) produced a percent recovery of 78.9% and 77.4%, respectively. These percent recoveries were similar to those obtained in FD and LD results from working solutions 2-5 of this study. While levels of 78% relative humidity were not encountered in this study, larger fractions of aerosols were most likely generated thereby increasing the surface area available for contact with water vapor causing a decrease in percent recovery.

Underestimations of TDI in both FD and LD filters were attributed to reactions with humidity since air was drawn in via a personal sampling pump. However, the loss of TDI was minimized in FD filters associated with working solution 1. This finding emphasizes the importance of immediate desorption for a specific concentration of TDI composed of a particular fraction of gas and aerosol phases. As the concentration of TDI increased, the particle size increased as well, causing agglomeration and increased surface area for water to react. Future studies are needed using the IAGS in combination with the modified closed-face cassette to determine how much volatilization was playing a role in the yields of percent recovery. More information is needed to determine the benefit of immediate desorption of TDI samples since four of the five working solutions did not show a significant difference. Additionally, humidity should also be monitored to determine if any small changes affect derivatization reaction yield. Using a similar approach as this study with opened-face cassettes, analysis for TDI is warranted to determine how much hydrolysis is occurring on the filter. Also a subset of samples should be analyzed by Fourier Transformed Infrared Spectroscopy to characterize any polymerization that may have occurred on the GFF.

CONCLUSION

The goal of this research was to determine the accuracy of FD and LD methods using a basic model of spray loading pure grade TDI. For example, no co-reactant (e.g., polyol) of TDI was present. The difficulties and challenges of sampling TDI were demonstrated in the results from a controlled setting. TDI is capable of partitioning between gas and particle phases, which has been suggested to play an important role in other studies. Due to the high vapor pressure and diffusion coefficient of TDI, volatilization and loss to the inside walls remains a concern.

Overall, FD and LD filters treated with aerosolized TDI consistently yielded relatively low amounts of TDI as compared to the theoretical amount. Since OSHA still prescribes opened-face cassette sampling with filter desorption arranged at the analytical laboratory, these results have broad implications in prudent practices for sampling worker exposures to TDI, especially since immediate desorption did not seem to facilitate the recovery of TDI. Additionally, a significant difference was observed only between FD and LD filters treated with TDI working solution 1 using the IAGS. The lack of a significant difference between FD and LD methods in working solution 2-5 should be further studied.

Many limitations inherent to this study suggest avenues for continued research. For example, one limitation of this study was the lack of particle size measurements and total concentration of the aerosol. Particle size may play a pivotal role in the quantitative determinations of TDI aerosol. Additionally, researchers have reported underestimations in the sampling of airborne TDI with the use of an opened-face cassette. The use of a modified closed-face cassette (as described earlier) would provide further knowledge on any loss of isocyanate. Together, particle size measurements and the use of a modified closed-face cassette would help eliminate uncertainty in TDI sampling. Finally, without controlling temperature and humidity in

this study, the effect of simultaneous collection of water vapor remains equivocal. An experimental matrix of different test conditions of humidity will allow determination of the rates of competitive reactions and their impact on analytical results.

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CHAPTER 5 – CONCLUSIONS

INTRODUCTION

The overall goal of this dissertation was to evaluate the performance of field and laboratory desorption of glass fiber filters following collection of airborne, aromatic isocyanate species, specifically methylene bisphenyl isocyanate (MDI) and toluene diisocyanate (TDI), using an aerosol generating system in a laboratory setting. Three different types of these aromatic isocyanates were used: technical and pure grade MDI, and pure grade TDI. In the first study (Chapter 2), the suitability of an isocyanate aerosol generating system (IAGS) for evaluating field (FD) and laboratory desorption (LD) techniques in isocyanate sampling using glass fiber filters (GFF) were investigated. This study included validation of the design and development of the IAGS to ensure consistent and accurate delivery of aerosolized isocyanates. FD and LD quantitative determinations of MDI (Chapter 3) were evaluated over five different concentrations of technical grade MDI obtained from a local Line-x company and pure grade MDI purchased from Sigma-Aldrich. The third study (Chapter 4) evaluated the effect of immediate desorption of GFFs following collection of airborne, pure grade TDI also purchased from Sigma-Aldrich.

SUMMARY AND SIGNIFICANCE OF EACH STUDY

An isocyanate aerosol generating system was designed and tested to provide a more realistic evaluation consistent with typical isocyanate exposure scenarios.

This aerosol generating system was the first one to be designed, built, and tested to evaluate the accuracy of isocyanate quantitation by FD and LD sampling methods after collection onto GFFs. The IAGS was validated with an experimental value of less than 1% error out of the spray nozzle using non-aerosolized water. Evaporation of aerosolized water was

observed, and was not included in the validation. When a relatively low concentration of MDI was spray loaded onto a sample set of filters (field desorbed only), IAGS test atmospheres were generated with a coefficient of variation of 8%, indicating a small variance relative to the mean value. . Experiments using varying amounts of toluene with a fixed amount of MDI showed that toluene did not interfere with 1,2-PP derivatization of MDI. Additionally, particle size measurements of IAGS aerosols were made with a GRIMM Dust Monitor 1.108. MDI aerosols (particles cm^{-3}) generated by the IAGS exhibited a unimodal size distribution present under 1 μm. The mass concentration shifted to larger particle diameters and a bimodal size distribution was observed that varied with concentration. The investigators concluded that the IAGS accurately and consistently delivered aerosols with sizes approximating those observed in field studies of spray polyurethane applications. Therefore, IAGS aerosols can be used to effectively to treat filters with aerosolized isocyanate to evaluate the accuracy of FD and LD sampling methods.

Field desorption of glass fiber filters yielded higher amounts of MDI than laboratory desorption, but significant underestimations of expected amounts of MDI were observed in the results from both desorption methods.

Overall, expected amounts of technical and pure grade MDI were significantly underestimated in both FD and LD sampling methods. However, test filters that were spray-loaded with aerosolized MDI solutions exhibited an overall greater percent recovery of the chemical when desorbed in the field immediately following collection rather than shipping the sample for laboratory desorption. Test filters were also liquid spiked with technical and pure grade MDI working solutions. No significant difference was observed in the percent recovery of MDI between FD and LD methods.

A significant three-way interaction between loading mechanism, loading concentration, and desorption method was modeled in this study. Using the general linear model, a multivariate assessment was conducted using the least square means of percent recovery to compare the mass of MDI collected by FD and LD methods at each loading mechanism and concentration. As a result, analytical results varied with the effects of loading concentration of MDI, desorption method, and loading mechanism (aerosol versus liquid).

This finding of a three-way interaction was not unexpected since researchers reported that FD methods consistently yielded higher amounts of MDI than LD methods in the field. Additionally, a difference in quantitative determinations was anticipated between spray and pipette loading of underivatized MDI onto filter. In contrast to the IAGS, pipette loading of filters was expected to yield higher amounts of MDI since a solution environment was created—facilitating derivatization by increasing mobility of both 1,2-PP and isocyanate. Chemical breakthrough of the GFF most likely occurred with pipetting; however, FD and LD results from filters pipette loaded with technical grade MDI still achieved 92% recovery.

Underestimations of airborne MDI in both FD and LD filters were attributed to aerosol size and subsequent reactions with water vapor in the air that simultaneously collected on the filter. However, the loss of MDI was minimized in FD filters since the extracting solvent dissolved both the derivatizing reagent and any un-reacted isocyanate, allowing the two to combine in solution and form a stable urea-derivative. The LD filters, instead, were not desorbed for at least a few days considering shipping time. MDI aerosols larger than 2 μm may have derivatized only a portion of the aerosol while the un-reacted portion was further exposed to humid air trapped inside the cassette after replacing the top cover and plugs.

Findings from this study have broad implications in protecting non-sensitized and sensitized workers through best practices and workplace standards. In this study, concerns were raised regarding the accuracy of glass fiber filter samplers and timing of desorption. This study addressed important knowledge gaps in isocyanate sampling. A more comprehensive sampling approach, which includes measuring environmental conditions, that is tailored to the process, or application, may be warranted for monitoring a worker's exposure to isocyanates in order to accurately interpret the results.

An intra-method comparison of quantitative determinations of technical and pure grade MDI revealed a significant difference.

Overall, quantitations of pure-grade MDI from filters that were pipette-, and spray-loaded were significantly lower than the results from the corresponding filters loaded with technical grade MDI. In this comparison, results from the same desorption method were compared instead of comparing the difference between the two desorption methods.

Estimates from simple differences (or partitioned analysis) of the LSMEANs from all four variables (grade, loading mechanism, loading concentration, desorption method) were used to evaluate statistical significance. Lack of significance was observed in FD and LD filters pipette loaded with working solutions 1 and 6, the lowest concentrations of MDI. Additionally, simple differences between LD LSMEANs associated with technical and pure grade working solutions 3 and 8, respectively, were not statistically significant. These two solutions were the second highest concentrations of MDI, which yielded a 4.9% difference in percent recovery.

The presence of other compounds in the technical grade MDI may account for these observed differences. Specifically, a bulk sample of STBL product was obtained from a local company that contained a proprietary component (25-45% w/w), and modified MDI (less than 10% w/w). The proprietary component may have insulated the MDI from competitive reactions.

Additionally, technical grade MDI contained modified MDI in addition to the monomer. The analytical laboratory did not distinguish between modified MDI and monomeric MDI. Therefore, signal or peak amplification of monomeric MDI may have occurred if these two compounds co-elute. Accordingly, analytical results of technical grade MDI may reflect a combined amount of monomeric and modified MDI.

Whether differences between technical and pure grade were significant or not, these results were practically relevant with serious worker implications since accuracy of MDI collection and analysis was related to composition of the product. An effect size greater than 4.9% (i.e., observed between working solutions 4 and 9) was still an important finding since this may correlate with a considerable amount of MDI mass depending on concentration encountered. As stated earlier, chronic low-level exposures (e.g., 2 ppb) are capable of causing the onset of sensitization.

Field desorption of glass fiber filters did not yield higher amounts of aerosolized TDI than laboratory desorption, but significant underestimations of expected amounts of TDI were observed in the results from both desorption methods.

In the third study, the investigator evaluated the FD and LD sampling methods for TDI using the IAGS. Using a similar experimental design, only aerosolized samples were collected. Findings from this study revealed only one statistically significant difference between FD and LD sampling methods, which occurred at the lowest concentration of TDI. Quantitative determinations of FD and LD filters after treatment with increasing concentrations of TDI did not vary with desorption.

Unlike in the MDI study, a two-way interaction (since only the IAGS was used) was not observed. Using the general linear model, a significant F-value of 5.26 (p-value <0.0001) was

detected in the omnibus, the main effect of desorption alone was not found to predict percent recovery with an F-value of 1.26 (Table 4-6). The F-value associated with concentration was 9.34, which produced a p-value < 0.0001. Accordingly, concentration was linearly related to percent recovery after accounting for desorption.

Collection of discreet droplets of pure grade TDI onto glass fiber filters using the IAGS significantly underestimated expected amounts of TDI. FD and LD filters treated with atomized TDI demonstrated a significant underestimation of recovered TDI compared to the theoretical amount across all working solutions.

TDI is capable of partitioning between gas and particle phases, which has been suggested to play an important role in other studies. Due to the high vapor pressure and diffusion coefficient of TDI, volatilization and loss to the inside walls remains a concern.

Findings from this study substantiate previous claims that while filters sample both gas and particle phases, mixing between the reagent and isocyanate is not always efficient. Underestimations of TDI in both FD and LD filters were attributed to reactions with humidity since air was drawn in via a personal sampling pump. However, the loss of TDI was minimized in FD filters associated with working solution 1. This finding emphasizes the importance of immediate desorption for a specific concentration of TDI composed of a particular fraction of gas and aerosol phases. As the concentration of TDI increased, the particle size distribution increased as well, which may have caused agglomeration, and even greater inaccessibility to the derivatizing agent.

CONCLUSION

The researchers confirmed in three studies the inherent difficulties and challenges of sampling both MDI and TDI. Sample results from collecting technical and pure grade MDI

showed that immediate desorption consistently yielded higher amounts of the chemical. However, immediate desorption of filters treated with aerosolized TDI did not show a significant difference from laboratory desorption. Collectively, both desorption methods significantly underestimated theoretical amounts of MDI and TDI. Furthermore, a three-way interaction was confirmed between loading mechanism, loading concentration, and desorption method in the MDI study, suggesting that even under optimal conditions for derivatization, isocyanate was still lost to competitive reactions with other nucleophilic agents that co-collected onto the filter. These studies also provided additional evidence on the accuracy of small amounts of MDI, which is essential to preventing sensitization or protecting sensitized workers. Finally, these studies provide further evidence that particle size measurements play an important role in quantitative determinations of isocyanates.

FUTURE STUDIES

Findings of this dissertation raised several questions for future investigations including:

1. *Investigation of dry MDI particle sizes emitted from the IAGS.* This study measured the intermediate particle sizes emitted from the IAGS, which were between the initial drop and dried particle phase. More data on the particle sizes after toluene evaporation would be beneficial in understanding derivatization kinetics and competitive reactions. The information from using vacuum oil as a surrogate measure of dried MDI suggests that smaller particle sizes of the isoscyanate may be formed after evaporation. The effect of particle size should be further explored with and without the presence of polyol to improve recommendations of sampler use and interpretations of results based on loss of isocyanate to inefficient mixing with derivatizing agent and subsequent loss to curing reactions.

2. *Investigation of FD and LD methods using monodispersed MDI aerosols.* The IAGS used in this research generated polydispersed aerosols with a particle size distribution that was fairly consistent with typical polyurethane spray applications encountered in the field. Selection of a monodispersed fraction from the initial IAGS aerosol size distribution would allow a more robust evaluation of the collection and derivatization of MDI aerosols in the size range of 2μm, which has been suggested as the upper limit for GFF sampling.
3. *Investigation of filters after sample collection using a scanning electron microscope.* Examination of the MDI sample surface topography and composition on the GFF after sample collection from both laboratory and field studies would provide valuable information regarding the relationship between quantitative determinations and dynamics of droplet and aerosol deposition, collection efficiency, and derivatization kinetics. One interpretation from this study suggested agglomeration of aerosols had occurred. Further research is needed to determine how much of the working area of the filter is being used. Micrographs from one study showed that contact of spray paint droplets with the fibers on GFFs was minimal. Further research is needed to quantitatively determine the magnitude of this lack of contact.
4. *Investigation of filter breakthrough using smaller pipette volumes.* The large decrease in percent recovery observed in filters that were pipette loaded with pure grade MDI working solutions, specifically between working solution 6 and 7, was attributed to chemical breakthrough of the filter. Further research is needed with lower pipette volumes of underivatized MDI to determine the impact of breakthrough on quantitative determinations.

5. *Investigation using different test conditions of temperature and humidity.* Significant differences were observed between the recovered amount of isocyanate and the theoretical amount. This difference was attributed to the loss of the analyte to competitive reactions, most likely with water vapor in the air. While previous studies have shown that there is no evidence that airborne MDI or TDI are hydrolyzed, once trapped on the filter such reactions can occur with simultaneous collection of other molecules containing active hydrogen atoms.
6. *Investigate the use of other nonpolar solvents when loading filters with technical and pure grade MDI.* In this research, a significant difference was identified between the two different grades of MDI. The technical grade MDI contained proprietary compounds that may have influenced dissolution of the NCO group in toluene. Further analyses using other nonpolar solvents should be performed to explore if the percent recovery of technical grade MDI were dependent on the state of its solvation. This will also provide information if the other constituents influenced percent recovery. Additionally, since modified MDI was one of the additives in the technical grade formula, spiked samples of pure grade MDI should be included in this study to determine if the response of monomeric MDI is elevated in the HPLC analysis.
7. *Investigate isocyanate loss to volatilization or to the walls of the cassette.* Using the modified closed-face cassette sampler, detection of MDI and TDI on the wall of the cassette can be performed. This information would be useful in helping understand the low percent recoveries identified in both FD and LD methods.
8. *Analyze GFFs for the presence of hyrdrolytic products and polymers.* This research focused on the detection and quantitation of the isocyanate-urea derivative. By-products

from competitive reactions are most likely stable, and therefore present on the filter. Filters could be analyzed using gas chromatography-mass spectrometry methods for the dectection of methylenediphenyl diamine and toluene diamine. Additionally, filters could be analyzed using Fourier transform infrared techniques for the presence of polymers, as well as unreacted NCO groups contained in any polyureas.

LIST OF ABBREVIATIONS

1,2-PP	1-(2-pyridyl)piperazine
ACGIH	American Conference of Governmental Industrial Hygienists
ANSI	American National Standards Institute
APS	Aerodynamic Particle Sizer
ARDS	Acute respiratory distress syndrome
BAL	Bronchoaveolar lavage
BAT	Biological tolerance value
BEI	Biological exposure indices
CAS	Chemical abstract Service
CDC	Centers for Disease Control
CDC	Center for Disease Control
CMD	Count median diameter
CPC	Condensation Particle Counter
CSU	Colorado State University
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FD	Field desorption
GC	Gas chromatography
GFF	Glass fiber filter
GSH	Glutathione
HAS	Human serum albumin
HCl	Hydrochloric acid
HDI	Hexamethylene diisocyanate
HLA	Human leukocyte antigent
HP	Hypersensitivity pneumonitis
HPLC	High-performance liquid chromatography
IgE	Immunoglobin E
III	International Isocyanate Institute
IL	Interleukin
IUPAC	International Union of Pure and Applied Chemistry
J	Joules
K	Kelvin
kg	Kilogram
LD	Laboratory desorption
LOD	Limit of detection
LOQ	Limit of quantitation
MAK	Maximum workplace concentration
MAMA	9-(methylaminomethyl) anthracene
MAP	1-(9-anthracenylmethyl) piperazine

MCE	Mixed cellulose ester
MDA	Methylenedianiline
MDI	Methylene bisphenyl isocyanate
MIF	Macrophage migrating inhibiting factor
MIP	Macrophage inflammatory protein
MMAD	Mean mass aerodynamic diameter
MMD	Mass median diameter
MMNTP	4-methoxy-6-(4-methoxy-1-napthyl)-1,3,5-triazine-2-(1-piperazine)
mol	Mole
MOPP	1-(2-methoxyphenyl) piperazine
NCO	Isocyanate functional group
NIOSH	National Institute for Occupational Safety and Health
NOAEL	No-observed-adverse-effect-level
NORA	National Occupational Research Agenda
OEL	Occupational exposure limit
OSHA	Occupational Safety and Health Administration
PAGE	Polyacrylamide gel electrophoresis
PBMC	Peripheral blood mononuclear cells
PEL	Permissible Exposure Limit
PPE	Personal protective equipment
PTFE	Polytetrafluoroethylene
PUF	polyurethane foam
PUR	Polyurethane
RADS	Reactive airway dysfunction syndrome
RAST	Radioallergosorbent testing
REL	Recommended Exposure Limit
RH	Relative humidity
RNA	Ribonucleic acid
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SMPS	Scanning Mobility Particle Sizer
SPRAY	Survey of Painters and Repairs of Autobody by YALE
STEL	Short Term Exposure Limit
TDI	Toluene diisocyanate
TIM1	T cell immunoglobin and mucin-domain containing molecules
TLV	Threshold Limit Value
TRIG	Total reactive isocyanate group
TWA	Time-weighted average
UK-HSE	United Kingdom Health and Safety Executive
URL	Upper reference limit
UV	Ultraviolet
WOHL	Wisconsin Occupational Health Laboratory