Final Progress Report

Dermal Exposure to 1,6-Hexamethylene Diisocyanate

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List of Abbreviations

ELISA – enzyme-linked immunosorbent assay; **FMOC** – 9-flourenylmethoxy-carbonyl; **GC-MS** – gas chromatography-mass spectrometry; **GFF** – glass fiber filter; **HDA** – 1,6-hexamethylene diamine; **HDI** – 1,6-hexamethylene diisocyanate; **HPFA** – heptafluorobutyric anhydride; **IOM** – Institute of Medicine; **K1** – keratin 1; **K10** – keratin 10; **K8** – keratin 8; **K18** – keratin 18; **LC-MS** – liquid chromatography-mass spectrometry; **MDL** – method detection limit; **MPP** – 1-(2-methoxyphenyl)piperazine; **NCI** – negative chemical ionization; **NIOSH** – National Institute for Occupational Safety and Health; **OSHA** – Occupational Safety and Health Administration; **PTFE** – polytetrafluoroethylene; **STEL** – short-term exposure limit; ***t*-Boc** – *t*-butyloxycarbonyl

Abstract

Skin is a major target for occupational chemical exposure and associated disease. Diisocyanates, used in applications involving polyurethane paints and plastics, are powerful sensitizers and a leading cause of occupationally induced asthma. Reaction of the isocyanate functional group with thiol groups of proteins such as keratins in skin may be involved in immune mediated responses and sensitization that could lead to development of allergic contact dermatitis (dermal-systemic effect) and also to asthma (airway-systemic effect). The relative magnitude of dermal versus inhalation exposure and the parameters that influence the extent of exposure by each route are not well understood. During this project, we have developed new efficient, sensitive, and specific techniques to measure 1,6-hexamethylene diisocyanate (HDI) monomer and its oligomers uretidone, biuret, and isocyanurate. To our knowledge, we are the first to synthesize and purifying urea derivatives of biuret and isocyanurate for use as standards and to quantifying HDI and its three oligomers in air, tape-stripped skin, and paint samples collected from occupationally exposed workers. The commonly available air samplers for HDI may significantly underestimate or produce biased results due to evaporation or due to the potential for vapor to adsorb to the filter, which is supposed to collect aerosols only. We evaluated these commonly used air sampling methods for HDI vapor and aerosol and determined the efficiency and accuracy of these methods for HDI under laboratory and occupational exposure settings. Our results show a potential for underestimation of HDI-based polyisocyanates when commonly used short-term dual-stage sampling is employed to measure airborne levels of HDI. Based on our results, a single-stage 37-mm polypropylene or polystyrene cassette should be employed when performing monitoring of inhalation exposure to HDI during spray-painting.

We also developed a method to measure 1,6-hexamethylene diamine (HDA), the hydrolysis product of HDI, which may serve as a biomarker of HDI exposure in the urine and blood of exposed workers. To our knowledge, we are the first to report quantification of HDA in plasma of workers exposed to HDI. As planned, we successfully recruited 48 spray painters (15 painters in North Carolina and 33 painters in Washington State) and performed 3 independent exposure assessment surveys during which we collected breathing-zone air, dermal, urine, and blood samples from all these workers. A preliminary analysis of the limited number of samples indicated a weak association between average dermal and breathing-zone concentrations of HDI monomer and oligomers during clear-coat application, multiplied by duration of application, and HDA concentrations in plasma. We are continuing to finalize all sample analyses and to investigate the relationship between the external exposure to HDI (both dermal and inhalation) and the levels of urine and blood HDA in this exposed spray-painter population.

Our initial strategy for synthesis of appropriate haptens for conjugation with carrier protein and for production of polyclonal antibodies against HDI-keratin adducts proved unworkable as a result of inability to remove the N^{α}-FMOC-protecting group from the modified amino acids following the addition step. However, a second approach was developed that involved coupling of *t*-BOC-protected 6-aminohexamethylene-isocyanate with the suitably protected 11-mers of keratin 1 and keratin 10, which gave good yields of the target epitopes and the production of monoclonal antibodies against these epitopes is in progress. An enzyme-linked immunosorbent assay (ELISA) method utilizing the produced monoclonal antibodies for detection of HDI-adducted keratin will be developed in order to measure HDI absorption and the potential systemic bioavailability *via* the skin. Once all the samples are analyzed, we will correlate the measured dermal concentration by the developed methods (ELISA and/or HDI extracted from the keratinized skin) with concentrations in other sentinel media (HDA in urine and plasma) and with breathing-zone concentrations of HDI and its oligomers. Together, these data will allow us to correlate dermal exposure to systemic exposure and to determine the significance of exposure through the skin.

A major accomplishment was the development of a model to predict dermal exposure to HDI monomer based upon breathing-zone concentration. The strong linear relationship between dermal exposure and product of breathing-zone concentration and paint time may form the basis on which new and more complex models can be built, including predictive models for dermal exposure to specific HDI oligomers resulting from deposition and models that consider the role of protective clothing and gloves, as well as other covariates that affect exposure. Once all sample analyses are completed, we will extend our investigation to determine how factors related to the work environment affect exposure to HDI monomer and its oligomers. In future studies, it will be crucial to use these new methods to characterize worker exposure to the specific diisocyanate species

in order to investigate the role of dermal exposure to HDI monomer and oligomers in induction of respiratory sensitization that may lead to occupational asthma. The methods developed and results obtained in the conduct of this study is valuable for understanding the role of dermal exposure as a vehicle for systemic exposure and for developing strategies to measure and minimize dermal exposure and, thus, to prevent adverse health effects.

Highlights/Significant Findings

The monomeric and polymeric isocyanates may differ significantly in their rates of absorption into tissue and their toxicity. Current sampling methods for measuring inhalation and dermal exposure to isocyanates cannot differentiate between the specific oligomers. During this project, we developed new efficient, sensitive, and specific techniques to measure HDI monomer and its oligomers uretidone, biuret, and isocyanurate. To our knowledge, we are the first to synthesize and purifying urea derivatives of biuret and isocyanurate for use as standards and to quantifying HDI and its oligomers in air, tape-stripped skin, and paint samples collected in the automotive refinishing industry. The developed tape-strip sampling provides a means to characterize the dermal exposure levels of HDI monomer and oligomers and investigate the penetration of each isocyanate into the skin. Uptake of HDI monomer, uretidone, biuret, and isocyanurate through the skin is likely to vary due to their differences in reactivity, volatility, solubility, and molecular weight. Because the skin has active metabolic and immune response properties, the residence time of the different isocyanates in the skin may affect how they are processed by the body, thereby leading to different levels of toxicity and even different health effects. As indicated, dermal exposures to HDI monomer and oligomers may play varying roles in respiratory sensitization. Therefore, these developed methods are crucial in order to explore quantitatively the contribution of each isocyanate species and exposure route and their relative contribution to the total body burden and to characterize these specific exposures in workers. In the future studies, it will be crucial to use these new developed methods to characterize worker exposure to the specific diisocyanate species in order to investigate the role of dermal exposure to HDI monomer and oligomers in induction of respiratory sensitization.

We also evaluated the commonly used air sampling methods for HDI vapor and aerosol and determining the efficiency and accuracy of these methods for HDI under laboratory and occupational exposure settings. Our results show that there is a potential for underestimation of HDI-based polyisocyanates when short-term dual-stage sampling is employed to measure breathing-zone concentration of HDI during the application of fast-curing clearcoat. Based on our results, a single-stage 37-mm polypropylene or polystyrene cassette should be employed when performing monitoring of halation exposure to HDI during spray-painting.

A major development from this study was a model to predict dermal exposure to HDI monomer based on breathing-zone concentration. The strong linear relationship between dermal exposure and product of breathing-zone concentration and paint time may form the basis on which new and more complete models are built, including predictive models for dermal exposure to specific HDI oligomers resulting from deposition and models that consider the role of protective clothing and gloves as well as other covariates.

To our knowledge, we are the first to report quantification of the HDA, which may serve as biomarkers of HDI exposure, in plasma of workers exposed to HDI. A preliminary analysis of the limited number of samples indicated a weak association between average dermal and breathing-zone concentrations of HDI monomer and oligomers during clear-coat application, multiplied by duration of application, and HDA concentrations in plasma. We are continuing to investigate the relationship between the external exposure to HDI (both dermal and inhalation) and the levels of urine and blood HDA in this exposed spray-painter population. A comprehensive understanding of the relationship between exposure route and systemic dose will help guide the direction of improving health and safety within the workplace where exposure to diisocyanates continues to remain a public health concern.

We observed that our initial strategy for synthesis of appropriate haptens for conjugation with carrier protein and for production of polyclonal antibodies against HDI-keratin adducts proved unworkable as a result of our inability to remove the N^{α}-FMOC-protecting group from the modified amino acids following the addition step. However, a second approach involving coupling of *t*-BOC-protected 6-aminohexamethylene-isocyanate with the suitably protected 11-mers of keratin 1 and keratin 10 gave good yields of the target epitopes. An ELISA method utilizing the produced monoclonal antibodies for detection of HDI-adducted keratin will be developed in order to measure HDI absorption and the potential systemic bioavailability *via* the skin. Once all the samples are analyzed, we will correlate the measured dermal concentration by the developed methods (ELISA and/or HDI extracted from the keratinized skin) with concentrations in other sentinel media (HDA in urine and plasma) and with breathing-zone concentrations of HDI and its oligomers.

Translation of Findings

The methods we have developed to measure HDI monomer and its oligomers uretidone, biuret, and isocyanurate are more sensitive and specific that the currently used monitoring methods for HDI. These methods will allow us to investigate the levels of HDI monomer and its oligomers in the breathing-zone and in the skin of automotive spray painters and characterize the exposure profiles, to correlate dermal exposure to systemic exposure and to determine the significance of exposure through the skin, and to investigate the relationships between the external exposure to specific diisocyanate species and measured biomarkers in blood and urine. The developed regression models may be used to predict dermal exposures to unprotected workers and may serve as the basis for a more complete statistical model that considers the protective effects of clothing and gloves. Statistical modeling of exposures will also allow us to characterize important differences in the exposure pathways of the each isocyanate species and to identify the chief determinants of inhalation and dermal exposure. This knowledge can then be used to recommend strategies and controls to prevent exposure. These models may further be used to assign inhalation and dermal exposure in retrospective epidemiological studies. There is even a possibility that these models may be extended to other sprayed chemicals in the automotive refinishing industry or to other industries where workers employ compressed air spray-painting. Ultimately, the results of our study will increase our knowledge and understanding of each route of exposure, which will be used to better protect workers.

Outcomes/Relevance/Impact

The methods developed during this project will allow us to characterize quantitative exposure profiles to HDI monomer and its oligomers in automotive spray painters, investigate the relationships between external exposure to individual diisocyanate species and biomarkers, investigate the contribution of each diisocyanate species and exposure route and their relative contribution to the total body burden, and identify important differences in predicted dermal concentration levels through regression modeling. The developed regression models may be used to predict dermal exposures in personal breathing zone measurements in unprotected workers and may serve as the basis for more complex statistical models that consider the protective effects of clothing and gloves to be considered. Statistical modeling of exposures will also allow us to characterize important differences in the exposure pathways of each isocyanate species and to identify the main determinants of inhalation and dermal exposure. In future studies, it will be crucial to use these new methods to characterize worker exposure to the specific diisocyanate species in order to investigate the role of dermal exposure to HDI monomer and oligomers in induction of respiratory sensitization. The gained knowledge can then be used to recommend strategies and controls to prevent exposure. These models may further be used to assign inhalation and dermal exposure levels in retrospective epidemiological studies. There is even a possibility that these models may be extended to other sprayed chemicals in the automotive refinishing industry or to other industries where workers employ compressed air spray-painting.

Scientific Report

Background

Skin is a major target for occupational chemical exposure and associated disease. Diisocyanates, used in applications involving polyurethane paints and plastics, are powerful sensitizers and a leading cause of occupationally induced asthma. Reaction of the isocyanate functional group with thiol groups of proteins such as keratins in skin may be involved in immune responses and sensitization that could lead to development of allergic contact dermatitis (dermal effect) and also to asthma (systemic effect). The relative magnitude of dermal versus inhalation exposure and the parameters that influence the extent of exposure by each route are not well understood.

This research project was designed to test two hypotheses: (1) the measured airborne concentrations of HDI in the workers' breathing-zones does not adequately reflect total systemic exposure and (2) a correlation can be established between airborne inhalation and/or dermal exposure and systemic dose measured by one or more biomarkers in skin, blood, and/or urine. Research into the deposition, absorption, and uptake of chemicals into and through the epidermis has been limited by lack of non-invasive methods to determine chemical-specific skin deposition and to investigate mechanisms of dermal toxicity. Previously, we have developed a non-invasive tape-strip technique for sampling layers of the epidermis for determination of a chemical concentration in the skin. In this project, we have developed and used air sampling and the tape-strip methods to quantitate and model dermal and inhalation exposure to HDI and its oligomers during spray-painting operations. Uptake of HDI monomer, uretidone, biuret, and isocyanurate through the skin is likely to vary due to their differences in reactivity, volatility, solubility, and molecular weight. Because the skin has active metabolic and immunological properties, the residence time of the different isocyanates within the skin may affect how they are processed, and, thus, lead to different levels of toxicity and even different health effects. If dermal exposures to HDI monomer and oligomers do indeed play varying roles in respiratory sensitization, then it is crucial that we can characterize these specific exposures in workers.

We have further developed a gas chromatography/mass spectrometry (GC-MS) method to measure the HDI hydrolysis product HDA, which may serve as a biomarker of HDI exposure, in urine and blood of exposed workers. We have extended our investigation to determine how factors related to the work environment affect exposure to HDI and its oligomers and to develop a mathematical model to predict dermal deposition for diisocyanates and other toxic materials in paint.

Specific Aims and Results

In Specific Aim #1, we describe the development and evaluation of a liquid chromatography/mass spectrometry (LC-MS) method for quantification of HDI and its oligomers in different sample media (1.1). We describe our results when comparing the commonly used air-sampling methods to quantify exposure to HDI-based polyisocyanates (1.2). We then compared dual-stage and single-stage sampling for HDI-based polyisocyanates and investigated the sampling bias associated with clearcoat curing time (1.3). Last, we describe the methods developed and used to measure HDA in blood and urine of the exposed workers (1.4). In Specific Aim #2, we describe the advances made towards the development of monoclonal antibodies for specific HDI-protein adducts using an ELISA. In Specific Aim #3, we describe our advances to develop predictive models for dermal exposure assessment.

Specific Aim #1: *To measure worker exposure to HDI during spray-painting operations. We will determine breathing-zone exposure to HDI by personal sampling, dermal exposure to HDI by noninvasive tape-stripping sampling of the stratum corneum, and systemic exposure by measuring the major metabolite of HDI, 1,6-hexamethylene diamine (HDA), in blood and urine.*

1.1. Development of Analytical Chemistry Method for Quantification of HDI and Its Oligomers in Breathing-Zone and Tape-Strip Samples

The monomeric and polymeric isocyanates may differ significantly in their rates of absorption into tissue and their toxicity. Current sampling methods for measuring inhalation and dermal exposure to isocyanates cannot differentiate between the specific oligomers. During this project, we have developed new techniques to

measure HDI-monomer and its oligomers (*i.e.*, uretidone, biuret, and isocyanurate) in the personal breathing-zone air samples, tape-strip samples collected from the exposed skin, and paint samples collected in the automotive refinishing industry. To generate analytical standards, urea derivatives of HDI, biuret, and isocyanurate were synthesized by reaction with 1-(2-methoxyphenyl)piperazine (MPP) and then purified. The urea derivatives were shown to be stable (degradation <2% per week at –20°C over a 2-month period) in occupational samples. The average recovery of HDI and its oligomers from tape was 100% and the limits of detection were 2 and 8 fmol/µl, respectively. The analytical method described in this study is specific (employing selective ion monitoring to identify individual HDI-based polyisocyanate species) and sensitive (capable of detecting trace amounts of isocyanates in different media). By synthesizing and purifying urea derivatives of biuret and isocyanurate for use as standards, we were able to determine the actual mass of collected polyisocyanates rather than the mass of pure product (*i.e.*, Desmodur N 3200) reported in other studies (Rudzinski *et al*, 1995; Maitre et al, 1996). The response ratios were linear over 2.3 orders of magnitude for each isocyanate, while a third-order polynomial equation was able to explain the response ratio for isocyanurate over 3.7 orders of magnitude. These dynamic ranges cover 100% of the levels in the occupational samples collected in this study.

These exposure assessments techniques and the LC/MS method for sample analysis were evaluated under field conditions on 13 automotive spray painters. The median breathing-zone concentrations of HDI, uretidone, biuret, and isocyanurate were 7.54, 6.78, 6.67, and 2,560 µg/m^3, respectively. The median dermal concentration levels of HDI, uretidone, biuret, and isocyanurate were 3.92, 11.9, 16.9, and 4,580 ng/mm^3, respectively. Isocyanurate was the main component of the measured exposure (N = 35 paint tasks), with median breathing-zone air and skin concentrations of 2.5 mg/m^3 and 3.7 µg/mm^3, respectively. A relationship was expected between dermal concentration and the product of breathing-zone concentration (intensity of overspray surrounding the painter) and paint time (duration of time in which overspray can deposit on the skin). Log-transformed dermal concentration correlated with the log-transformed product of breathing-zone concentration and paint time for HDI ($r = 0.78$, SE = 0.97, $p < 0.0001$) and isocyanurate ($r = 0.77$, SE = 1.0, $p < 0.0001$), respectively. We did not find a significant correlation between dermal concentration and the product of paint concentration and paint time for HDI ($p = 0.0894$) or isocyanurate ($p = 0.390$). This underscores the important role played by factors other than the concentration in the paint, such as airflow in the booth and painter positioning, in determining both breathing-zone concentration and dermal concentration. The sensitivity of the analytical method allows detection of HDI and its oligomers in air (sampling at 1 l/min for 15 min) at concentrations that are over 700 times lower than the NIOSH ceiling limit for HDI (140 µg/m^3) or the Oregon short-term exposure limit (STEL) for biuret and isocyanurate (1 mg/m^3). The Oregon STEL was exceeded in 71% of the samples, with the highest isocyanurate air concentration (17.8 mg/m^3) being over 15 times greater than the recommended limit.

The results demonstrate that the methods developed during this project are very efficient, sensitive, and specific to quantify HDI-monomer and its oligomers on samples collected in occupational exposure settings. This part of the project has been published in the Scandinavian Journal of Work, Environment and Health (Fent et al. 2006) and a second manuscript has been submitted for publication (Fent et al. *submitted*).

1.2. Comparison of Air Sampling Methods for HDI-based Polyisocyanates

The commonly available air samplers for HDI may significantly underestimate or produce biased results due to evaporation or due to the potential for vapor to adsorb to the filter, which is supposed to collect aerosols only. Thus, we evaluated the commonly used air sampling methods for HDI vapor and aerosol and determining the efficiency and accuracy of these methods for HDI under laboratory and occupational exposure settings. This part of the project has been published as a Master's technical report (Thomasen 2007; a manuscript for publication is under preparation).

During these experiments we determined the recovery of HDI vapor generated in the laboratory with several sampling devices [open and closed-face single and dual-stage 37-mm polystyrene and polypropylene cassettes, IOM cassettes (steel and plastic), OSHA Method 42, and IsoChek® cassettes] as well as the reactivity of HDI with different sampling materials. In addition, the collection efficiencies of these sampler types were investigated in a spray-painting facility during application of clear coat.

In the laboratory experiments we observed that the cassette material had an influence on the collection of HDI and that sampling losses may occur due to the reactivity of HDI with sampling materials. These results also indicated that the reaction of HDI with sampling materials is amplified by the presence of a non-polar

solvent (i.e., toluene) in the environment. The results from the field experiments indicated that the clear coat used during spray-painting activities affects the collection efficiency of the sampling device. These results also suggest that HDI oligomers may polymerize on the surface of the polytetrafluoroethylene (PTFE) filter of dual-stage sampling cassettes. However, in the field setting, the cassette material did not seem to impact the sampling efficiency as greatly as it did in the laboratory setting.

Based on the results of these experiments, a single-stage 37-mm polypropylene or polystyrene cassette when performing field sampling for HDI during spray-painting in the auto-body industry should be used. The use of the single-stage sampler will reduce the possibility of polymerization of HDI monomer and oligomers. However, aerosol and vapor phases cannot be separated with single-stage sampler. Additional laboratory and occupational sampling experiments need to be performed with all sampling devices and different clear coats to fully evaluate their reliability.

1.3. Comparison of Dual-stage and Single-stage Sampling for HDI-based Polyisocyanates

Given our concern that HDI-based polyisocyanates may polymerize on the surface of a PTFE filter during dual-stage sampling of atmospheres containing automotive paint overspray, particularly when fast curing clearcoat is sprayed, we evaluated the sampling performances of dual-stage and single-stage filter cassettes in the occupational environment. The dual-stage cassettes were constructed of 37-mm polystyrene. The first stage held a PTFE filter (Millipore Corp., Billerica, MA) with 5-µm pore size designed to collect HDI aerosol. The second stage held a glass fiber filter (GFF) (SKC Inc., Eighty Four, PA) with 1-µm pore size designed to collect HDI vapor. The GFF was impregnated with derivatizing agent by adding 400 µl of 43 mg/L MPP in toluene to the filter and allowing the toluene to evaporate before placing the filter in the cassette. A cellulose pad (Millipore) was used to support the GFF. The single-stage cassettes were identical to the dual-stage cassettes except the PTFE filter (first stage) was not included. An impinger filled with 15 mL of derivatizing solution was used as a reference. Workers sprayed either fast or slow-curing clearcoat in the vicinity of the samplers, which sampled air at 1 L/min. After 15 min of sampling, the filters were submerged into 5 mL of derivatizing solution. The filter and impinger samples were then analyzed by LC-MS for quantitation of HDI, uretidone, biuret, and isocyanurate.

Comparisons were made for the dual-stage cassettes between fast and slow-curing clearcoat and for fast-curing clearcoat between single and dual-stage sampling. Although the sample size was small, significant differences were detected. For dual-stage sampling, significantly ($\alpha = 0.05$) higher levels of biuret and isocyanurate were collected when slow-curing clearcoat was sprayed than when fast-curing clearcoat was sprayed. When fast-curing clearcoat was sprayed, single-stage sampling collected significantly ($\alpha = 0.05$) greater amounts of uretidone, biuret, and isocyanurate than dual-stage sampling. These results suggest that there is a potential for underestimation of HDI-based polyisocyanates when short-term (≤15 min) dual-stage sampling is employed during the application of fast-curing clearcoat. Consequently, single-stage sampling may be a more reliable method for monitoring atmospheres consisting of HDI-based polyisocyanate aerosols and vapor, as it is less prone to errors and gives more consistent results than dual-stage sampling. There is much more knowledge to be gained concerning measurement bias, however, by paired personal dual-stage and single-stage sampling during automotive spray painting.

The results provide critical information about the performance and suitability of different samples to monitor inhalation exposure to HDI and its oligomers and this information will be used in conjunction with the biological monitoring to help model the dermal exposure process and enhance our understanding of the fate of HDI after contact with skin. A manuscript is under preparation.

1.4. 1,6-Hexamethylene Diamine (HDA) in Blood and Urine

For this project, we recruited 48 spray painters (15 painters in North Carolina and 33 painters in Washington State) and performed 3 independent exposure assessment surveys in which we collected breathing-zone air, dermal, urine, and blood samples from all these subjects.

A total of 417 urine samples from the 48 spray painters were collected. Each spray painter provided one urine sample before the start of work (pre-shift sample), a variable number of samples during the workday, and one sample just prior to leaving work (post-shift sample). HDA, the hydrolysis product of HDI, in urine is analyzed as a heptafluorobutyric anhydride (HFBA) derivative by GC-MS in negative chemical ionization (NCI) mode using selective ion monitoring at m/z 448. The method detection limit (MDL) for HDA is 0.08 ng/ml. Creatinine level in urine is also determined. To date, we have completed the analysis of in 329 samples

collected from 44 spray painters. The highest measured HDA concentration in urine was 10.12 μg/l, and the overall mean ± standard deviation was 0.34 ± 0.82 μg/l. The pre-shift HDA concentration in the urine varied from <MDL to 7.75 μg/l with a mean of 0.31 μg/l and the post-shift HDA concentration in urine varied from <MDL to 10.12 μg/l with a mean of 0.46 μg/l.

HDA in the plasma of exposed workers was also measured as a biomarker of exposure to HDI. Blood samples were collected at the end of the work-shift from 46 of 48 spray painters recruited for this study. A total of 112 plasma samples were obtained. HDA in the plasma fraction was quantified using a modified version of the protocol developed by Rosenberg *et al.* (2002). The samples were analyzed by GC-MS in NCI mode using a fused silica capillary column (DB-5MS, 30m x 0.25mm x 0.25 μm) with methane as reagent gas. The mass fragment used in identification was m/z 448 for HDA. The MDL for HDA was 0.02 ng/ml plasma. The highest measured HDA concentration in the plasma was 0.92 ng/ml, and the overall mean ± standard deviation among the workers was 0.10 ± 0.16 ng/ml. There was no significant difference in HDA levels in the plasma between those sampled in Washington State and participants sampled in North Carolina. There was no significant difference in plasma HDA levels between visits 1–3 in North Carolina ($p = 0.85$), although there was a moderate decrease in plasma levels for visits 1–3 in Washington ($p = 0.05$). There was a weak association between average dermal and breathing-zone concentrations of HDI monomer and oligomers during clear-coat application, multiplied by duration of application, and HDA concentrations in plasma (r^2 = 0.06-0.15 and r^2 = 0.08-0.21, respectively).

To our knowledge, this the first study to quantify HDA in plasma of isocyanate-exposed workers. A manuscript describing the analytical method to measure HDA in the plasma of exposed workers is under preparation. We are continuing to investigate the relationship between the external exposure to HDI (both dermal and inhalation) and the levels of HDA in urine and blood in this exposed spray-painter population. A comprehensive understanding of the relationship between exposure route and systemic dose will help guide the direction of improving health and safety within the workplace where exposure to diisocyanates continues to remain a public health concern.

Specific Aim #2: *To investigate dermal penetration by analysis of HDI-protein adducts in successive layers of the epidermis obtained by the non-invasive tape-strip sampling, in order to assess the contribution of dermal uptake to the overall systemic burden. To this end we will combine our tape-stripping technique with assays for specific HDI-keratin adducts.*

To determine the importance of dermal exposure in developing a systemic response to HDI we will analyze samples of tape-stripped epidermis and sera from a population of exposed spray painters for specific HDI-protein adducts using an ELISA. Likely targets for adduct formation are the cysteine residue in keratin head sequences of keratin 1 (K1) and keratin 10 (K10), and arginine and lysine residues in head sequences of keratin 8 (K8) and keratin 18 (K18). To develop the ELISA, we considered the adduct structures to be the 6-aminohexamethylene carbamoyl derivatives of the respective residues. The initial strategy was to incorporate for synthesis of appropriate haptens was to couple the *t*-BOC-protected 6-aminohexamethylene-carbamoyl-modified, N^{α}-FMOC-protected amino acids into an appropriate 11-mer keratin head sequence by automated oligopeptide methods for conjugation with carrier protein. This route proved unworkable as a result of difficulty removing the N^{α}-FMOC-protecting group from the modified amino acids following the addition step. However, a second approach involving coupling of *t*-BOC-protected 6-aminohexamethylene-isocyanate with the suitably protected 11-mers of K1 and K10 gave good yields of the target epitopes. Monoclonal antibodies are currently being produced against keyhole limpet hemocyanin conjugated modified K1 and K10 11-mers. We are scheduled to receive the monoclonal antibodies in December 2007 after which the ELISA will be developed and the tape-strip samples collected from the 48 spray painters will be analyzed. The results obtained will yield insight into the correlation of dermal exposure both with dermal dose and the levels of HDA in urine and blood. A manuscript describing the synthesis of the modified peptides and production of monoclonal antibodies is in progress.

Specific Aim #3: *To investigate the correlation between measured dermal, inhalation, and systemic exposures using mixed-effects linear-regression analyses to determine how physical and structural parameters of the work environment affect exposure to HDI, to model dermal deposition rates using computational fluid dynamic (CFD) exposure modeling, and to predict impact of strategies for minimizing exposure.*

A dermal exposure computer model was completed based on a key paper by Asset and Pury (1954) indicating that aerosol deposition on body hair is a key determinant of dermal exposure. This led to the formulation of an in-house computer code to estimate the dermal deposition of HDI during spray-painting operations. The model predictions compared favorably with field data, and the results have been published (Flynn *et al*, 2006).

Given these advances, we proceeded to model dermal and inhalation exposure to specific polyisocyanates. A major development from this study was a model to predict dermal exposure to HDI Monomer based on breathing-zone concentration. To accomplish this we collected personal breathing-zone samples of 7 workers who spray-painted automobiles and collected tape-strip samples immediately following completion of each task. Tape-strip samples were collected from six different 10 cm^2 sites of the skin (i.e., right and left volar and dorsal arm and right and left dorsal hand). Like the air filters, the tape-strips were analyzed by LC-MS (Fent *et al.* 2006) for quantitation of HDI monomer. The amount of HDI on each site of skin was determined by summing the levels collected with 3 successive tape-strips. These levels were averaged across all sampled sites to determine the mean dermal exposure level per task. Our analysis was restricted to workers who did not wear protective clothing or gloves. Efforts were made to avoid collecting skin that had been exposed through splashing or product transfer. Thus, deposition was the primary mechanism of exposure.

Log-transformed dermal exposure was correlated with the log-transformed product of breathing-zone concentration and paint time ($r = 0.68$, $SE = 0.28$, $p = 0.0009$). This strong correlation suggests that any air or tape-strip sampling biases were either relatively constant across all workers and paint tasks or directly related to one another. In this model, breathing-zone concentration represents the intensity of exposure surrounding the worker during the paint-task, while the paint time represents the duration of exposure. The formula relating exposure to the product of intensity and duration is widely used in environmental and occupational epidemiology. These results provide additional evidence that HDI exposure to unprotected skin may be modeled using breathing-zone concentration as a predictor. Because the same method was used to analyze both the air and tape-strip samples, we can be confident in the comparability between measurements. The strong linear relationship between dermal exposure and product of breathing-zone concentration and paint time may form the basis on which new and more complex models are built, including predictive models for dermal exposure to specific HDI oligomers resulting from deposition and models that consider the role of protective clothing and gloves, as well as other covariates.

We will extend our investigation to determine how and/or why exposure modeling differs between the different isocyanates and identify which factors are the most important determinants of exposure. Determination of factors related to the work and work environment that affect dermal and inhalation exposure to HDI and determination of correlation of measured dermal exposure with measured environmental exposure concentrations of HDI and its measured metabolite in urine and blood using mixed-effects linear regression analyses will be completed after all samples have been collected and analyzed.

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Thomasen, J: [2007] Laboratory and Field Comparisons of Air Sampling Methods for 1,6-Hexamethylene Diisocyanate, MSPH Technical Report, Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill.

Publications

Journal Articles:

1. Fent K, Nylander-French LA, Ball LM, Gold A, Jayaraj K: [2006] Tape-strip sampling to measure dermal exposure to 1,6-hexamethylene diisocyanate, Scandinavian Journal of Work, Environment and Health 32(3):225-240.
2. Flynn MR., Koto Y, Fent K, Nylander-French LA: [2006] Modeling dermal exposure – an illustration for spray painting applications. Journal of Occupational and Environmental Hygiene 3:475–480.

Dissertations/Theses:

Thomasen, J: [2007] Laboratory and Field Comparisons of Air Sampling Methods for 1,6-Hexamethylene Diisocyanate, MSPH Technical Report, Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill.

Inclusion Enrollment Report Table

Study Title: Dermal Exposure to 1,6-Hexamethylene Diisocyanate	
Total Enrollment: 48	**Protocol Number:** UNCSPH 01-1353
Grant Number: R01 OH007598	

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Sex/Gender				
	Females	Males	Unknown or Not Reported	Total	
Hispanic or Latino	0	8	0	8	**
Not Hispanic or Latino	0	40	0	40	
Unknown (Individuals not reporting ethnicity)	0	0	0	0	
Ethnic Category: Total of All Subjects*	0	48	0	48	*
Racial Categories					
American Indian/Alaska Native	0	1	0	1	
Asian	0	1	0	1	
Native Hawaiian or Other Pacific Islander	0	0	0	0	
Black or African American	0	4	0	4	
White	0	31	0	31	
More than one race	0	11	0	11	
Unknown or not reported	0	0	0	0	
Racial Categories: Total of All Subjects*	0	48	0	48	*

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Unknown or Not Reported	Total	
American Indian or Alaska Native	0	0	0	0	
Asian	0	0	0	0	
Native Hawaiian or Other Pacific Islander	0	0	0	0	
Black or African American	0	0	0	0	
White	0	0	0	0	
More Than One Race	0	8	0	8	
Unknown or not reported	0	0	0	0	
Racial Categories: Total of Hispanics or Latinos**	0	8	0	8	**

* These totals must agree.
** These totals must agree.

Inclusion of Children

Not applicable.

Materials Available for Other Investigators

No data or research materials are available to be shared with other investigators at this time.