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Aliphatic Isocyanate Skin Transferability In Automotive Refinishing

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ALIPHATIC ISOCYANATE SKIN TRANSFERABILITY IN AUTOMOTIVE REFINISHING

By

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ABSTRACT

Isocyanate (NCO) skin contact may contribute to sensitization and the development of isocyanate asthma. Unbound NCO can persist on polyurethane (PU) spray-coated car parts and other surfaces, after appearing dry. Whether human isocyanate skin exposure can result from handling such surfaces remains unclear. To assess NCO transfer potential to human skin from surfaces recently sprayed with aliphatic isocyanate coatings used in collision repair work, quantitative surface and skin wipe sampling for total NCO was performed on test panels sprayed with such coatings and on skin samples obtained from participants who had rubbed the recently dried surfaces. 18 workers in 5 auto body shops participated. Surface and skin samples were prepared following NIOSH method 5525 (modified for skin samples) and isocyanate species (HDI, pHDI, pIPDI and total NCO) analyzed using high-performance liquid chromatography (HPLC) with ultraviolet (UV) and fluorescence (FLD) detectors. Quantifiable unbound NCO species were detected on 84.2% of all sprayed surfaces sampled when initially considered dry (n= 38 samples). A significant ($p < 0.001$) decay in free NCO was observed over 24 hours. For all 104 skin samples obtained after contact with recently dried coatings only 6.7% (7 out of 104) had detectable quantities of free NCO. The 7 positive samples were all obtained at the initial sampling time (t_0) and had a geometric mean of 0.016 μg NCO cm^{-2} (range: 0.002-0.88 μg NCO cm^{-2}). Only 1 of the 12 (8.3%) skin samples obtained after compounding was positive for free NCO. All study control (pre-contact) skin samples were negative. Limited transfer of free NCO from surfaces with detectable NCO levels to the skin of workers handling them was documented. The risk of substantial human isocyanate skin exposure from contact with the dry appearing (yet potentially semi-cured) isocyanate coatings evaluated in this study appears to be low, although other products and tasks may pose a more substantial dermal NCO exposure potential.

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1. INTRODUCTION

Aliphatic isocyanates are reactive low molecular weight organic compounds which contain at least one -N=C=O (NCO) functional group. These agents are readily used to generate ultraviolet (UV) and chemical resistant polyurethane (PU) coatings applied to products of the automotive, aerospace, and construction industries.[1-3] Despite recent improvements in the industrial hygiene controls in place at many worksites including better ventilation systems and employee respirator use, along with the use of less volatile isocyanate-containing products, these potent chemical sensitizers remain one of the most common causes of occupational asthma.[4] Repeated exposure to isocyanates can lead to sensitization, with spray painters at a particularly high risk.[5-7] Once sensitization has occurred, even minute levels of subsequent airborne exposure can give rise to asthmatic attacks, a major health problem affecting up to one quarter of the workers exposed to this class of chemicals.[4, 8] In addition to the respiratory route of exposure, dermal contact with these products likely contributes to isocyanate sensitization, thus warranting a better understanding of the possible sources of skin exposure.[4, 5, 9]

It has been demonstrated that recently coated car surfaces (with which auto body workers frequently come into contact) can contain unbound isocyanate (NCO) species even after appearing to have dried, suggesting that such isocyanates may be a potential source of skin exposure when workers touch these car parts.[10] Of note, the aforementioned investigation primarily performed sampling using a surface qualitative method and did not evaluate worker skin isocyanate exposure. As opposed to surface NCO analysis, skin isocyanate exposure assessment has proven far more challenging given the inadequacy of existing methodologies (removal techniques are problematic for reactive chemicals and may lead to significant losses)

variety of isocyanate products in use, uncertainty regarding NCO skin absorption rates, as well as the complicated and sporadic nature of the tasks performed by workers in the field of automotive refinishing.[5, 11, 12] Limited field studies to date have suggested that skin NCO exposure may be associated with not only spray painting, but also common paint-related tasks (e.g. compounding, unmasking, wet sanding and others) on surfaces recently coated with isocyanate-containing paints.[7, 13] However, these prior studies had limited controls in place (e.g. pre and post-task sampling) and they collected only a small number of skin samples. Paired surface and skin sampling in tandem are essential in demonstrating any potential transfer of unbound NCO from a dried auto body surface sprayed with isocyanate-containing paint while undergoing task-related tactile manipulation by a worker.

Whether contact with surfaces that recently were coated with isocyanate paints and appear dried can result in transfer of free isocyanate species to human skin is an important question that has yet to be investigated. Contact with such surfaces without use of protective clothing and gloves is common and may be an important and unsuspected source of isocyanate exposure and risk factor for isocyanate asthma. If NCO species can be transferred to human skin from diisocyanate-coated surfaces, understanding the factors (e.g. curing time) and tasks that modify the risk of such exposure should markedly facilitate preventive strategies to reduce worker exposures.

1A SPECIFIC AIMS:

The specific aims of this study were to:

i) Evaluate the extent to which free aliphatic isocyanates may be transferred to the skin of auto body shop workers from automotive panels painted with aliphatic isocyanate coatings and dried

under standard working conditions.

ia) Evaluate the transferability of free isocyanate species from a representative group of coatings to human skin once the coatings have dried (time t_0).

ib) Evaluate the transferability of free isocyanate species from selected coatings to human skin as a function of time after drying (t_0 to t_i) to define conditions that reduce risk of exposure.
ii) Determine the extent to which free aliphatic isocyanates can be transferred to skin during a routine end-use task typically performed by auto body shop workers on recently coated parts.

The primary end-use task that will be evaluated is compounding of dry clearcoat. The findings should clarify whether free NCO species can be transferred to human skin from recently applied aliphatic isocyanate coatings, and also help target effective preventive strategies.

2. BACKGROUND AND SIGNIFICANCE

2A OCCUPATIONAL ASTHMA:

Throughout the Occident, occupational asthma (OA) is now the most frequently reported work-related disease of the lung.[14-16] Clinically, the condition involves variable limitation of airflow following occupational exposure to substances that can cause asthma.[17] Asthma has been reported to be the result of exposures from the workplace in as many as 16.3 % of patients with an adult onset.[18] Additionally, there is evidence that greater asthma severity may be associated with patients with exposures of an occupational nature.[19] Given the high price paid by patients with OA, both in terms of lost quality of life and productivity, this disease category poses a major public health threat. In the U.S. during 1996 alone, the cost of OA was

conservatively estimated to be on the upwards of $1.6 billion and as the prevalence of this condition continues to rise the financial burden will likely follow suit.[20, 21]

OA is classified into two main divisions including: irritant-induced and allergic asthma.[8, 22] The former condition which was initially referred to as reactive airway dysfunction syndrome (RADS) and typically develops within one day of a worker being directly exposed to a substantial amount of a hazardous chemical such as hydrogen sulfide or diisocyanate.[8, 14] On the other hand, allergic OA involves a latent period during which immunologic sensitization to the workplace allergen occurs gradually over the course of weeks to years.[1, 17] Additionally, after a sensitized individual is exposed, symptoms can occur shortly after exposure or be delayed up to 6-15 hours beyond that point. Such a delay in respiratory symptoms often poses a considerable challenge to patients and clinicians alike with respect to making connections between work-related exposures and OA symptoms.[23] Of the few hundred industrial chemicals already implicated as work-related asthmagens, diisocyanates in particular have been among the most commonly reported causes of allergic OA.[1, 12, 24] Globally current projections for the development of sensitization and subsequent OA among workers exposed to diisocyanates range from 5% to 10%, however, even higher estimates on the order of 25% have also been reported.[4, 24, 25]

2B <u>ISOCYANATES:</u>

Isocyanates are reactive organic compounds which contain at least one unsaturated -N=C=O functional group.[6, 26] These chemicals are used to generate PU products when they exothermically react with active hydrogen atoms in the multiple hydroxyl groups of other cross-linking reagents such as polyfunctional alcohols termed polyols.[23, 27] Isocyanates of both the

aromatic (i.e. containing at least one benezene ring) and aliphatic (i.e. saturated non-aromatic hydrocarbons) form are further classified based upon their number of free reactive functional groups; where compounds can have either one (monoisocyanate) two (diisocyanate monomer) or three or greater (polyisocyanate) NCO groups.[28] Furthermore, these monomeric isocyanates can be combined through condensation reactions to give rise to polymeric NCO species. [28] In order of increasing volatility, aromatic diphenylmethane diisocyanate (MDI) and toluene diisocyanate (TDI) as well as aliphatic hexamethylene diisocyanate (HDI) are of the highest commercial use within the PU industry and these agents are also among the most common causes of OA.[4, 24, 29, 30]

The collision repair industry is one of the occupational sectors where workers face the greatest risk of NCO sensitization and the subsequent development of OA.[8, 31] At present in the U.S. alone, there are approximately 35,600 auto body refinishing shops employing almost 224,000 workers, which constitutes a considerable population with the potential for isocyanate exposures and their debilitating pulmonary sequelae.[32] In this setting, isocyanates are ubiquitous and found primarily within the cross-linking PU hardener component of various coatings. The painter mixes together the isocyanate component with the solvent base (polyol) of the primer or clearcoat depending on the desired coating type to be sprayed.[21] Due to their superior gloss retention, plus UV light and abrasion resistance properties which lead to a better paint job, aliphatic as opposed to aromatic formulations are the isocyanates of choice.[6, 28]

Traditionally, the respiratory tract has been considered to serve as the fundamental route of worker NCO exposure with spray painters at greatest risk. [2, 25, 31, 33, 34] Therefore in recent times, manufacturers have worked to attenuate the volatility of isocyanate-containing products in the hopes of diminishing the extent of NCO vapor inhalation and in turn the risk of sensitization

and the development of asthma. [21, 26] As with other PU manufacturers and end-users, auto body shops have tried to combat NCO inhalational exposures through various measures. These attempts include employing ventilation controls, respiratory personal protective equipment (PPE) and by using lower volatility aliphatic isocyanates such as the polymeric species of hexamethylene diisocyanate (pHDI) (most commonly biuret, uretidone and isocyanurate)[9] and polymeric isophorone diisocyanate (pIPDI) which collectively contribute over 99% of the total NCO groups found in current automotive refinishing paints, with the more volatile monomers of these two isocyanate species contributing less than 1%.[2, 4, 9, 35, 36] Nevertheless, even polymeric species of diisocyanates can be potent asthmogens.[28] Furthermore, the incidence as well as prevalence rates of isocyanate-induced asthma have persisted among this population of end-users affecting up to a quarter of exposed workers.[4, 24, 37, 38] Therefore beyond the respiratory route, dermal NCO exposure may considerably factor into both isocyanate sensitization and asthma, focusing attention on possible sources of skin exposure. A brief description of the auto body repair process is beneficial for understanding the potential routes of diisocyanate exposure among this workforce.

2C AUTO BODY SHOP PRACTICES:

After an automobile accident, body shops generally fix cars in three main stages (EPA 1996). First structural manipulations are made to bent areas of the car frame while electrical and mechanical repairs are performed. Thereafter, the altered car body panels are restored using filler materials composed of polyester resins if needed or they are replaced, then welding, sanding and grinding occurs to leave the damaged car surfaces smooth and flush. Painting procedures comprise the final phase of the repair process at which time painters and body technicians are at

the greatest, but not the sole, risk for diisocyanate exposures. Although spray painters have been found to be at highest direct inhalational and dermal exposure risk, the small size of many collision repair shops and curing of parts that can occur outside of the spray booth enhances the risk of bystander exposures as well.[2, 31] Aside from the actual application of the various coatings several other tasks are associated with the painting process including: paint mixing, cleaning of spray equipment, removal of the painter's tape, sanding, buffing, polishing and compounding of the dried car body.[34] Unlike the original spray painting performed on the body of a new car at the factory (where coatings are cured through baking at temperatures approaching 400 °F (204 °C) in body shops cars have cloth and leather fabrics plus plastic components such as wiring internally that cannot withstand heat in excess of 150 °F (66 °C) which is the reason that diisocyanate hardeners are used to greatly facilitate the curing of coatings at lower ambient conditions.[6]

New or sanded car parts initially receive an application of one or more layers of primer with or without a sealer coating, both of which frequently contain diisocyanates.[34] The subsequent layers of basecoat and clearcoat are referred to as the topcoat.[6] The basecoat finish which generally contains no diisocyanate additives is the pigmented coating which gives the painted surface its desired color, whereas the clearcoat is a transparent product readily mixed with diisocyanates and applied to protect the underlying finishes.[6] Solvents ranging from acetone to toluene are widely used to dissolve the diisocyanates added to the various coatings.[31]

Prior to the early 1990s auto body refinishing paints contained extensive concentrations of organic solvents, but recently there have been increasing governmental and industrial attempts to limit the volatile organic compound (VOC) levels found within these products.[6, 39] To accomplish this objective, collision repair shops nationwide have slowly begun to substitute

waterborne basecoats in place of solvent containing basecoats. At present, only two districts in California have put in place official regulations making the switch mandatory, however, such requirements are expected to sweep across the nation over the next decade.[40] Europe has already made the transition to waterborne paint products to a far greater extent than the U.S. nevertheless, comparable diisocyanate exposures still persist even among workers in shops using waterborne products given the fact that these paint jobs still require the same clearcoats and primer coatings which contain identical diisocyanate hardener additives as those found in the traditional solvent based finishes.[2] Therefore, waterborne paint systems may help to lower worker exposures to solvents and reduce the VOC emissions into the atmosphere yet they in no way provide additional protection from diisocyanate exposures, since they pertain primarily to the basecoat rather than the clearcoat and primer components. If anything, the manufacturing of diisocyanates and their use in automotive refinishing paints continues to increase.[21]

2D DIISOCYANATE INDUCED ASTHMA AND SUSPECTED IMMUNOPATHOGENESIS:

All of the diisocyanates are considered to be low molecular weight species, yet the HDI monomer and its polymers have the lowest respective molecular weights of this class, each well below 600 daltons.[9, 15] The potential for respiratory as well as skin sensitization is of particular concern with compounds such as the diisocyanates whose molecular weights fall under 1,000 daltons which has particular implications for the suspected development of diisocyanate induced OA.[6] Given their miniscule structures, diisocyanates are considered to be haptens that require a carrier protein with which to react to form an antigen complex capable of eliciting an immune response.[11, 15] In humans, the carrier proteins of the respiratory epithelia, serum and skin which have been observed to most readily join with diisocyanates include albumin and keratin.[27]

Furthermore, it has been proposed that covalent binding between the diisocyanate hapten compounds and their protein conjugates may be of key importance to the cascade of events that result in sensitization and OA.[28]

A number of immunological hallmarks distinguish diisocyanate induced asthma from atopic asthma. Atopic asthma involves an offending environmental allergen (usually a high molecular weight compound) which is large enough to induce the sensitization and subsequent asthmatic response without the need to combine with a carrier protein.[24, 41] Unlike the latter condition, patients with diisocyanate induced OA tend to demonstrate diminished serum levels of specific IgE immunoglobulin, a combination of T helper (Th) Type 1 and 2 activity, as well as a $CD8^+$ T cell response.[42, 43] Research to date indicates that diisocyanate exposures including those to MDI and HDI monomer and polymers result in the production of IgG immunoglobulins specific to their conjugate antigen formed by a diisocyanate and carrier protein.[23, 44] Despite the lack of evidence to support a strong IgE immunoglobulin involvement in the majority of diisocyanate induced asthmatics, the presence of specific IgG seems to be at best only an exposure marker.[26] Nevertheless, human evidence has suggested that OA due to diisocyanates involves features of both the innate as well as the adaptive immune systems.[41]

After sensitization to diisocyanates extremely minute quantities of NCO species, on the order of 1 part per billion (ppb), can trigger an asthmatic response.[45] Of great concern is the fact that such low levels of exposure are present at many work sites that use diisocyanate containing chemicals and that still meet all exposure limits currently in existence.[11] The respiratory inflammation associated with both atopic and diisocyanate induced OA stems from an influx of inflammatory cells including eosinophils, mast cells and neutrophils, as well as the priming of lymphocytes and release of pro-inflammatory cytokines in the airway mucosa.[23, 24, 37] Even after

the diagnosis of diisocyanate asthma is made and the worker is removed completely from the chemical stimulus, symptoms of diisocyanate OA may persist and be triggered by a variety of non-specific chemical factors as the result of chronic inflammatory and fibrotic alterations in the composition of the airways and altered patterns of mucus secretion.[24, 42] Therefore, since isocyanate exposures in workers seldom give rise to overt irritations, as well as the fact that long-term pathologic sequelae frequently accompany this diagnosis, prevention is a critical strategy for diisocyanate induced OA.[11, 23, 29]

2E DIISOCYANATE EXPOSURE ASSESSMENT:

A variety of methods have been described in the literature for measuring the levels of diisocyanate exposures, predominantly for those of an airborne nature. Ambient air concentrations within the worksite, particularly collision repair shops have been routinely studied and suggest that bystanders in the general vicinity of diisocyanate use may also be exposed.[7, 21] Both quantitative as well as colorimetric qualitative methods have been described for gauging the presence of free diisocyanate species on numerous work surfaces as well as upon coated auto body parts.[10, 35] Internal doses of diisocyanates following controlled human exposures have been investigated via analysis of conjugates of protein and diisocyanate within broncheoalveolar lavage (BAL) fluid and serum.[46] In addition, elevated hexamethylene diamine (HDA) levels have been used as a proxy urinary biomarker for detecting HDI monomer exposures in workers.[7] However, none of the currently available biomarkers is particularly well validated or practical.

To date the majority of research on diisocyanate-induced asthma has focused upon the respiratory route of exposure.[2, 5] Nevertheless, increasing evidence suggests that the skin may

also be an important route of free NCO exposure leading to sensitization after all. HDI and its biuret and isocyanurate oligomers along with TDI and MDI have all been shown to induce respiratory hypersensitivity in guinea pigs.[47-49] A murine model has also been described which mimicked the intense mixed Th Type 1 and 2 mediated airway inflammatory response observed in humans following dermal sensitization to HDI and subsequent intranasal challenge with the HDI antigen complex.[50] In humans, several studies have also implicated epicutaneous diisocyanate exposures as a potential route for sensitization and in turn OA.[7, 35, 51] One study in particular found that differences in spray paint worksite ventilation quality had little bearing upon the considerable diisocyanate exposures sustained by employees at the different shops, again suggesting a possible role of dermal exposure.[2]

Current methods available for the investigation of dermal diisocyanate exposure are limited and challenging given that these agents tend to be components of products that consist of complex mixtures of various chemicals which can markedly alter the uptake and binding of diisocyanates to dermal proteins.[11] The difficulty is further amplified when sampling in a collision repair shop setting where employee tasks can widely fluctuate depending upon the day's work, several diisocyanate-containing products are used, as well as inconsistencies in the utilization of personal protective equipment (PPE).[12] Even when PPE is worn, dermal patch sampling under gloves has indicated that penetration of diisocyanates to the surface of the skin can occur.[34]

Qualitative and quantitative skin wipes have also been described in the literature, yet these methods are particularly dependent upon the absorption rates of diisocyanates on human skin which largely remain unknown, especially for complex mixtures.[13, 35] All cutaneous sampling strategies are extremely time-sensitive since they depend upon the transient amount of

free diisocyanate species present upon the skin, however, some methods may be somewhat more hardened against this obstacle than others. For instance, tape-stripping can be used to remove consecutive layers of the stratum corneum which may capture NCO species that have been absorbed but have not yet reacted with the proteins of the skin.[9] Dermal biopsy has also been used to collect deeper tissue layers of human skin after controlled exposure to diisocyanates.[46] In the context of auto body shops, curing times of various diisocyanate products have been shown to vary widely which opens the door to the transfer of unreacted NCO species from an inanimate surface to the skin of workers.[10]

3. METHODS

3A TERMINOLOGY:

Paint system technical data pamphlets frequently use the terms *dried* and *cured* interchangeably to mean that the sprayed auto body surface is ready for the next work task. In this study, only *drying* will be defined in that manner. On the contrary, the term *cured* will describe the state where all functional NCO groups have been consumed in the formation of a polymeric network. Therefore, when a surface is indeed fully cured there will be no unbound isocyanate molecules present or available to be transferred through contact with the dried part.[6, 10] The terms *unbound* and *free* will be used as synonyms throughout this paper to describe such unreacted isocyanate (i.e. NCO) groups in chemical moieties which are not yet joined to the polymeric network.

3B AUTO BODY REFINISHING OPERATIONS AND OPPORTUNITIES FOR DERMAL EXPOSURE:

After structural repair and surface preparation, auto body refinishing culminates in various painting procedures (e.g. paint mixing, application of coatings, cleaning of spray equipment, removing masking tape from painted cars, dry or wet sanding of auto body panels coated with isocyanate-containing paints that have dried, compounding and detailing).[7, 34, 35, 52] The latter tasks in particular can involve skin contact with dry, but potentially semi-cured surfaces, posing a risk for dermal NCO exposure.[21] In general, a trio of product types is sequentially sprayed on the car surface after each has been allowed to dry in order to perform the body restoration. A primer coating with or without a prior sealer is first applied to the auto body surface on the shop floor followed with a drying procedure under infrared lamps and or in the open air. Then the automobile is generally moved into a ventilated spray booth where the remaining coatings will be applied and then dried within that enclosure with a baking cycle around 140 °F (60 °C). Thereafter, a basecoat is sprayed which gives the car surface its actual color and finally a protective topcoat (i.e. the clearcoat) is laid down.[31] While the number of applied coats of these product types can vary depending upon the given job and shop practices, each of these coatings with the exception of the basecoat usually contain an isocyanate hardener or activator component.[10, 31, 34] In addition, drying times also fluctuate depending upon the given product type, drying method and shop. In general, drying times can range from over 5 minutes between coats to 2-4 hours for tasks such as sanding and can be up to 16 hours for final detailing prior to delivery of the refinished car back to the owner.[10]

3C STUDY POPULATION AND AUTO BODY SHOPS:

Eighteen auto body shop workers (Table 1) without previous isocyanate sensitization or current asthma, from 5 refinishing shops (Table 2) in the New Haven, CT area were recruited to participate in this experimental exposure assessment which employed intra-participant controls. The study protocol was approved by the Human Investigation Committee of the Yale University School of Medicine and Yale-New Haven Hospital. Additionally, informed written consent was obtained from each participant. Recruited workers completed a questionnaire (Appendix A) which included items related to respiratory and skin symptoms, medical and occupational history, as well as use of PPE.

3D PAINT APPLICATION AND SURFACE PREPARATION:

In total, painters sprayed 42 standard (10.5 x 15 cm) steel test panels (DuPont M-5832; Wilmington, DE) with aliphatic isocyanate-containing coatings and allowed them to dry according to manufacturer/shop specifications (i.e. baking, heating under infrared lamps and or air drying). In order to realistically gauge the risk of worker dermal NCO exposure, a range of 5 product brands routinely used in the study shops was evaluated resulting in a total of 10 different coating types (i.e. 5 primers and 5 clearcoats). Each test panel was sprayed with its final applied coating being 1 of 5 different paint brands of primer (n=12) or clearcoat (n=30). More specifically, if a primer was to be tested then that test panel would only be sprayed with the primer. On the other hand, to evaluate specific clearcoats, test panels first underwent application of the underlying primer and basecoat layers in accordance to shop practices. Thereafter the clearcoat was sprayed since this is the standard sequence followed in refinishing, as previously described. These painted surfaces were then dried by either baking in a booth followed by air drying (n=32), heating with infrared lamps then air drying (n=6), or air drying alone (n=4). All

panels were sprayed and dried next to actual jobs in each shop. An overview of collected surface and skin samples is shown in Table 3.

3E SAMPLING OF DRIED PAINTED SURFACES AT THE INITIAL TIME POINT (T_0):

After test panels were sprayed, shop personnel were asked to determine the point at which each painted surface was suitably dry for handling and work (time t_0). At that time, distinct cells of a fixed surface area (16 cm^2) outlined by an overlaid template (Appendix B) specific to each panel were wipe sampled by the investigator who used fresh nitrile gloves (KC500 Kimberly-Clark Corp; Roswell, GA) for each sample. Wipe samples were collected for quantitative isocyanate analysis and semi-quantitative (i.e. qualitative/colorimetric) analysis, using 5 x 5 cm polypropylene glycol (PPG) impregnated quantitative wipes (Colormetric Laboratories, Inc; Des Plaines, IL), as done previously by Bello et al.,[10] and 2.5 x 3.0 cm qualitative surface SWYPEs™ also from CLI, respectively as previously described.[10, 35] Both types of sampling were performed concurrently to evaluate the degree of surface contamination with free NCO species in order to compare with subsequent skin samples that were collected following contact with different cells of said dried isocyanate paint-coated panels.

A quantitative surface pad was wiped within its designated test panel window by a gloved researcher following a standardized rubbing protocol, in which the investigator used his thumb, index and middle finger to grip the pad and firmly wipe the entirety of each cell with 10 seconds of vertical strokes followed by 10 seconds of horizontal strokes. The used surface wipe was then deposited within its own scintillation vial containing 10 ml of derivatizing solution (5 $x10^{-4}$ M 1-9-anthracenylmethyl-piperazine (MAP) dissolved in acetonitrile) sealed and immediately placed with the other samples within a cooler containing ice packs (≈ 38 °F / 3 °C) and shipped

overnight to the laboratory. Once samples were received in the laboratory they were kept in a freezer until preparation and analysis. Bulk samples of the diisocyanate-containing hardener used in each tested product were also collected for analysis along with field blank samples of unused surface quantitative and qualitative wipes. Chemical analysis was performed for numerous isocyanate species including: monomeric hexamethylene diisocyanate (HDI) several higher oligomeric species of HDI commonly found in polymeric hexamethylene diisocyanate (pHDI) paints in auto body shops (uretidine dione, biuret, isocyanurate and diisocyanurate) and polymeric isophorone diisocyanate (pIPDI). The sum of oligomeric species of HDI was reported as pHDI. In bulk products between 65-95% of the total isocyanate content in pHDI is contained in a few major analytes, including biuret, isocyanurate, and occasionally uretidine dione among others. Total NCO was calculated as the sum of all isocyanate species (HDI, pHDI, pIPDI) per sample.

Each qualitative surface pad was wiped within its specific test panel cell according to the same rubbing protocol described above. The SWYPE^{TM} pad was then immediately inspected for any color change indicative of the presence of surface free NCO. As previously described, color intensity was rated on a 0 to 5 scale with 0 representing no color change, 1 light orange and 5 (deep red) being the highest intensity.[10] Additionally, all color intensity scores were rated by the same investigator. The sampled surface area size, wiping technique, frequency and precautionary measures (e.g. new nitrile gloves for each sample and identical grid templates unique to each panel) against cross contamination were identical between the surface quantitative and qualitative sampling procedures with all samples collected on site by the same investigator.

3F SAMPLING OF RUBBED SKIN SURFACES AT THE INITIAL TIME POINT (T_0):

Prior to rubbing procedures, each participant's hands along with the investigator's were washed in a standardized manner using soap (5 Star® X-treme Hand Cleaner #5995; Scottsdale, Arizona). After the participants' hands were completely dry, a published tape-strip sampling method[53] was performed to obtain the skin samples, which were analyzed as previously described.[10, 13] Beginning with a designated control finger, a tape-strip with dimensions 2 x 2.5 cm of Cover-Roll® adhesive tape (Beiersdorf AG; Hamburg, Germany) was applied and smoothed upon the worker's finger tip by the freshly gloved researcher using forceps (that had been cleaned with methanol). Thereafter, a nontoxic washable marker was used to outline the applied tape-strip in order to allow for an identical placement of the subsequent tape-strip. The initial strip was left in place for exactly 1 minute after which it was peeled away using the cleaned forceps and deposited in a vial containing 10 ml of derivatizing solution (5×10^{-4} M 1-9-anthracenylmethylpiperazine (MAP) dissolved in acetonitrile). A second tape-strip was immediately applied in the same manner and also left in place for 1 minute, before being removed and transferred into the same vial as the corresponding first strip. Collectively, the tape-strips were used to sequentially remove 2 layers of stratum corneum. In an effort to minimize the risk of cross-contamination new nitrile gloves were donned by the researcher and new forceps were used between each finger tested. In addition to the 2 tape-strips which were collected from an un-rubbed finger to serve as a negative control, 2 additional tape-strips were transferred directly into the derivatizing solution without skin tape-stripping to serve as field blanks.

Following the collection of a negative control sample (2 tape-strips), for each specified finger, study participants were then asked to contact the dried test panels with the volar aspect of their fingers. Each worker was asked to rub a particular finger upon a dried test panel window for a total of 20 seconds (10 seconds with a vertical motion of the finger tip region corresponding to

the distal to intermediate phalanx, depending upon the size of the worker's hands, immediately followed by another 10 seconds using a horizontal motion) with sufficient force to blanch the terminal perimeter of the digit. As described above, two tape-strips were then applied and left in place one after the other for 1 minute apiece before being peeled off and deposited together in a scintillation vial unique to each sampled finger. This procedure was repeated using an additional yet different finger on a separate panel cell for duplicate sampling. At the completion of the sampling, the participant's hands were again washed prior to returning to work.

Tape-stripping was the primary method used to sample workers' skin. Nevertheless, to compare dermal quantitative methodologies, a limited number of skin wipe samples (n=16) were also obtained in addition to tape-strip sampling after participants performed the rubbing protocol. PPG impregnated 5 x 5 cm quantitative skin wipe pads (Colormetric Laboratories, Inc; Des Plaines, IL) were used according to a previously published methodology.[13] More specifically, prior to hand washing, sampling first involved measuring the length and width dimensions of each of the workers' finger pad rubbing surfaces over which the wipe sampling would be performed. After the rubbing protocol for each specific finger, the entire volar area distal to the distal interphalangeal (DIP) joint was wiped by the gloved investigator with 10 seconds of vertical followed by 10 seconds of horizontal strokes to cover the entirety of the given finger pad.

3G SURFACE AND SKIN SAMPLING OVER TIME (T_0-T_3):

Two of the shops involved in the initial time point (t_0) sampling as discussed above were selected for additional surface and skin sampling over time. A primer was tested at one of the shops while a clearcoat was evaluated at the other. Once the coatings were considered to have

dried, 2 different auto body shop workers at each site underwent quantitative skin tape-strip sampling in duplicate (2 different fingers), at 4 different time intervals (i.e. at approximately t_0=0 hrs, t_1=1.5 hrs, t_2=3 hrs and t_3=24 hrs post-dry time). Corresponding quantitative surface sampling was also performed at each time point for both products in duplicate in order to assess the relationship between the presence of unreacted surface aliphatic diisocyanates and transfer to human skin. In addition, quantitative control finger and qualitative surface wipe samples were collected at t_0 and t_3.

3H PERI-COMPOUNDING SURFACE AND SKIN SAMPLING:

In this phase of the study transfer of free NCO species to human skin was assessed prior to and following the common shop task of compounding. Compounding is a procedure performed late in the automotive refinishing process which involves buffing the dried outermost layer of clearcoat with polishing paste to remove flaws caused by dust particles, which can involve skin contact with dried yet incompletely cured isocyanate sprayed surfaces.[21] Two workers from 3 shops performed compounding for a period of approximately 2.5 minutes on their respective test panels that had been previously sprayed with clearcoat, allowed to dry and then had surface sampling performed on them as above. In addition to quantitative and qualitative surface wipe sampling, duplicate skin tape-strip samples from 2 fingers were obtained after rubbing the compounded surface using the study rubbing protocol, which again included control finger samples as well. Study samples were all processed and analyzed as described above.

3I STATISTICAL ANALYSIS:

Tests for normality, descriptive statistics, t-tests and multivariate regression analyses were performed with SAS® version 9.2 statistical software (SAS Institute; Cary, North Carolina). Given the data fit a lognormal distribution, the geometric mean (GM) and geometric standard deviation (GSD) were used to characterize data distribution. Surface quantitative sample data was used to construct a model in which the dependent variable was ln(μg surface total NCO). In order to achieve the most parsimonious model in the multivariate regression analysis, a backward elimination strategy was employed whereby all collected study variables were initially present then extracted one at a time based on their extent of failure to reach a p-value of 0.05. Significance for all statistical tests performed in the study analysis was assessed based on an α of 0.05. In addition, individual sample values for polymeric hexamethylene diisocyanate (pHDI) ≤ the limit of detection (LOD) were substituted with ½ LOD as done previously.[13]

4. RESULTS

4A SAMPLE COATING PRODUCTS AND PANEL PREPARATION CHARACTERISTICS:

Ten different coatings were evaluated through the study, however the percentage of aliphatic isocyanate contained within each varied as the hardener component of these products differed from one another. Overall, for both product types (i.e. primers and clearcoats) the percentage of each coating that was comprised of NCO-containing hardener relative to the other paint ingredients (i.e. the hardener ratio) ranged from 0.087-0.333. The primers alone had hardener ratios which ranged from 0.087-0.200, while the clearcoat hardener ratios ranged from 0.200-0.333. Average primer drying time (again, deemed by shop personnel) was 59 minutes (range: 30-105 minutes). For primers, the average total time between the final paint application

and the execution of worker skin sampling was 151 minutes (range: 54-385 minutes). The clearcoats had an average drying time of 110 minutes (range: 37-255 minutes) with an average total time between clearcoat application and the execution of skin sampling being 192 minutes (range: 75-355 minutes). In addition, average drying conditions for sprayed study test panels included: 0.5 hours at 143.7 °F (62 °C) when baked, 0.33 hours at 119.5 °F (49 °C) when heated with infrared lamps and 2.8 hours at 81.3 °F (27 °C) when air dried. The mean relative humidity during the drying period was 47.9% (range: 25-59%).

4B DRIED SURFACE AND RUBBED SKIN SAMPLING AT THE INITIAL TIME POINT (T_0):

Unbound NCO species were detected in 32/38 (84.2%) of quantitative wipe samples obtained from surfaces sprayed with isocyanate-containing coatings that appeared fully dried at the initial time point (t_0) (Table 4). Surfaces sprayed with clearcoat (n=28) showed significantly ($p < 0.008$) higher levels of free NCO/cm^{-2} (geometric mean of 0.139 μg NCO/cm^{-2}) than surfaces coated only with primer (n=10; geometric mean of 0.063 μg NCO/cm^{-2}).

A total of 80 skin samples were obtained from the auto body shop workers at t_0 (immediately after the painted surface appeared dry) (Table 4). Ten different coatings (primers and clearcoats) were each surface sampled and tested by at least 2 different auto body workers, with duplicate skin samples taken from each individual for every product (64 tape-strip skin samples). Quantitative skin wipe samples were also obtained (n=16) plus 20 control samples (Table 3). For the skin samples obtained at t_0 only 5 out of 64 (8%) tape-strip samples and 2 out of 16 (13%) wipe samples had positive isocyanate species on the skin (Table 4). All control samples were negative. Thus only 7 out of 80 total (8.7%) skin samples obtained after contact

with the coatings shortly after they appeared dry were positive for quantifiable free NCO on the skin and these were all obtained at time t_0.

Substantial variability was observed among the 7 positive skin samples. The 2 skin samples with the greatest total NCO were tape-strip samples and were collected from a single participant after contact with the clearcoated surface. Of note, the baked and dried test panel corresponding to these 2 samples had a 37 minute drying time which was the lowest of any panel evaluated in the study, as well as a relatively low total time between clearcoat application and the actual execution of the sampling procedures, which was 83 minutes after the final coating had been sprayed. The other 5 positive skin samples (3 clearcoats and 2 primers) had markedly lower levels of total NCO detected and little in terms of overt patterns (e.g. extremes in drying times, type of coating or skin sampling method) to account for their variability (Table 5).

Multivariate regression with a backward elimination strategy was performed during the study analysis phase to identify predictive factors for surface NCO contamination upon panels being deemed dry (Table 6). The final model yielded an R-squared value of 0.92 and multiple interactions were identified. At the initial sampling point (t_0) it was modeled that for either clearcoat or primer spray painted test panels (painted at 72 °F (22 °C) in 50% relative humidity) increasing amounts of NCO-containing hardener results in reduced surface contamination with free isocyanate groups (Figure 1). Nevertheless, this attenuation of automotive surface unbound NCO groups with ascending hardener ratio appears to be considerably more prominent in primer than clearcoat products so that overall, clearcoats result in a higher degree of surface contamination relative to primers. In addition, another interaction was noted between humidity and temperature conditions during the air drying period for surfaces sprayed with primer using a 20% hardener ratio. In this model for ambient drying temperatures up to approximately 73 °F (23

°C) higher humidity levels during the drying period correlate with lower amounts of free NCO on the automotive surfaces at the point in which they are first considered dry. However, at air drying temperatures in excess of 73 °F the opposite pattern is predicted based on the model whereby ascending relative humidity during the drying period correlates with increased surface free NCO contamination (Figure 2).

4C SURFACE AND SKIN SAMPLING OVER TIME (T_0-T_3):

Two shops which underwent surface and skin sampling at t_0 were re-selected to undergo more extensive sampling over time. A primer was selected at one of the shops while a clearcoat was chosen at the other and each coating type was tested by 2 different auto body technicians per shop at 4 different time intervals after the coatings were considered to have dried (i.e. at approximately t_0=0 hrs, t_1=1.5 hrs, t_2=3 hrs and t_3=24 hrs) (Table 3). Ten samples were taken from each participant (2 different fingers at each time point, plus 2 controls) following the same approach and surface rubbing protocol as described above. Also as before, paired quantitative test panel surface sampling was performed at each time point for all products, in duplicate, in order to assess the relationship between the presence of un-reacted surface aliphatic isocyanates and transfer to human skin.

Levels of free NCO detected on the surface of the painted panels decreased significantly ($p < 0.001$) over time for all paints tested (Table 7). Furthermore, the primers cured more quickly than the clearcoats (Figure 3). At approximately 24 hours post-drying, levels of total NCO were low, yet they still contained detectable isocyanate species (Table 7). Of the 32 skin samples obtained from the auto body workers after rubbing the painted surfaces (8 at each time point) only 1 skin sample (3.1%) showed detectable free NCO. This sample was obtained at time t_0, and

was also included in the 7 positive skin samples noted above. Thus no free NCO was detected on skin samples obtained at times after t_0. Overall, only 7 out of the 104 (6.7%) total skin samples obtained in the non-compounding phases of the study were positive for free NCO, and these 7 were all obtained around t_0, shortly after the surface was deemed dry.

4D PERI-COMPOUNDING SURFACE AND SKIN SAMPLING:

The presence of free NCO species on human skin was evaluated following the routine shop task of compounding dried surfaces that had been coated with clearcoat. The painted surfaces were sampled before and after compounding, both qualitatively and quantitatively. Six workers were recruited (2 from each of 3 shops) and performed compounding using their standard procedure (and compound products) for a period of approximately 2.5 minutes on their respective test panels that had been previously sprayed with clearcoat and allowed to dry. A total of 24 tape-strip samples were obtained from 6 workers, 12 before and 12 after rubbing the compounded surface using the study rubbing protocol as previously described (Table 3).

Paired qualitative and quantitative wipe sampling both showed detectable levels of free NCO on all 6 test panel surfaces before compounding (Figures 4 and 5 and Table 8). The pre-task levels of unbound NCO detected quantitatively on the 6 surfaces decreased following compounding (from geometric mean 0.176 to 0.042 μg total NCO cm^{-2}) although this change was not statistically significant ($p > 0.157$). However, all but one of the 6 qualitative SWYPE™ samples showed an increase in the surface levels of unbound NCO following compounding relative to the baseline samples prior to the task (Figures 4 and 5).

Only 1 out of 12 (8%) of skin samples obtained after compounding was positive for free NCO. The 12 skin samples obtained before the compounding task were all negative for free NCO (Table 8).

5. DISCUSSION

Residues of aliphatic isocyanates were detected and quantified on recently painted test panel surfaces beginning when the paint products were deemed dry. Consistent with research to date, in the current study the amount of unbound NCO recovered from the surfaces decayed over time.[10] Most notably, the presence of unbound NCO on skin which had touched coated surfaces, after they were deemed dry, was detected in only a few of all the samples analyzed (6.7% of all skin samples). The limited positive skin samples were all obtained shortly after the product was considered dry (time t_0) and had relatively low levels of total free isocyanate. None of the 24 skin samples obtained from painted surfaces at later time points after the surfaces appeared dry (i.e. 1.5 to 24 hours) showed detectable amounts of free NCO. Additionally, only one of the quantitative skin samples obtained after compounding showed any detectable unbound NCO.

5A STUDY FINDINGS IN RELATION TO THE CURRENT LITERATURE:

Previously published data showed detectable unbound isocyanate species on freshly painted auto body surfaces for hours to days after appearing dry.[10] This prior investigation predominantly involved qualitative surface sampling of NCO sprayed car parts with quantitative sampling of only 2 auto body surfaces longitudinally. Our current study-wide clearcoat samples collected at t_0 had a geometric mean of 0.139 μg total NCO cm^{-2} which is similar to the previous

study's findings where samples from the clearcoated car parts immediately following their baking period yielded a geometric mean of 0.109 μg NCO cm^{-2}. Among the rest of the samples taken over time our investigation found slightly higher surface isocyanate contamination levels. For instance, the geometric mean of quantitative samples taken from a car part at approximately 2.5 hours after surface baking was 0.083 μg NCO cm^{-2} in the prior publication, while our reported geometric mean for clearcoated surface samples at t_2 (i.e. 3 hours) post-dry was 0.216 μg NCO cm^{-2}. The quantitative samples taken at 24 hours in the earlier study resulted in a geometric mean of 0.015 μg NCO cm^{-2}, whereas our geometric mean for t_3 (i.e. ≈ 24 hours) post-dry was 0.051 μg NCO cm^{-2}. Such small inter-study differences are expected and could be due to a variety of factors including but not limited to differences in: clearcoat brands and in turn dissimilar hardener ratios, individual shop practices, surface areas coated and sampled or environmental factors (e.g. ambient temperature or humidity) or variation during preparation and sampling of the various parts. Still, given the scale of NCO measurement employed, both studies had rather similar findings overall.

In addition to the marked persistence of free isocyanate groups on automotive surfaces well beyond the drying time (a finding reinforced by the current investigation) which raised the concern that recently painted surfaces could be a potential source of dermal NCO exposure, another study describing skin exposure to aliphatic isocyanates in auto body workers demonstrated detectable free NCO on the skin of a small number of workers performing tasks such as compounding, un-taping and different forms of sanding.[13] However, our current more extensive quantitative skin sampling data performed through this study unexpectedly showed minimal isocyanate skin exposure from contact with (i.e. rubbing) dry recently spray painted surfaces prior to and after compounding. There are a number of potential explanations for this

paucity of documented surface to dermal transfer of NCO species which are briefly highlighted below. Nevertheless, we believe the current skin sampling data likely reflects the risk of dermal exposure related to contact with recently coated surfaces under the conditions tested. The prior study included a relatively small number of skin samples from workers performing non-spray painting tasks and importantly did not include baseline comparison samples obtained prior to task execution. Furthermore, workers did not all first wash their hands in a standardized manner, as was done in this study. In the current investigation, the predominantly negative skin sampling results could not be attributed to painted surfaces containing no detectable NCO, as the corresponding dry sprayed surfaces were also sampled. Furthermore, the dermal as well as the surface NCO levels measured in this study were still in the same general range as those previously reported.[10, 13]

5B POTENTIAL GENERATION OF THERMAL DEGRADATION PRODUCTS THROUGH THE COMPOUNDING TASK:

An unexpected finding was the incongruence between paired qualitative and quantitative isocyanate sampling results obtained from the same coated surfaces immediately preceding and following compounding. A decrease in total free NCO was detected quantitatively but an increase in free NCO using the qualitative SWYPEs was noted comparing levels before and after compounding. In the automotive repair setting, increasing attention is being directed at the generation of thermal degradation products during certain processes (e.g. welding, cutting, grinding, sanding or polishing) which involve heating car parts covered with dried PU coatings.[2, 54] Unlike NCO exposures from paint mixing or spray painting where the worker is susceptible to specific isocyanates found within the given coating formulation, thermal

degradation processes can result in the formation and emission of a wide array of novel and complex mixtures of isocyanates as the result of secondary reactions (e.g. chain breaking, isomerization and or dehydrogenation).[54, 55] Such species can be difficult to quantify using current HPLC methodologies. Prior research has demonstrated the emission of isocyanate species into the air in immediate proximity to the source of friction during sanding of coated dry car panels.[54] This finding highlights the potential for a reintroduction of NCO species upon auto body surfaces undergoing such work and perhaps even a possible source of dermal isocyanate exposure and sensitization.

In the current study it is possible that compounding generated NCO thermal degradation products that were detected with qualitative sampling yet undetected through the quantitative analysis. Such thermal degradation products if indeed transferred to skin would still have been difficult to detect with only the same HPLC methodology available for skin analysis. Nevertheless, further studies are needed to evaluate the extent to which compounding or other tasks (e.g. sanding) can generate NCO thermal degradation products that could pose a risk of unsuspected isocyanate exposure.

5C POTENTIAL DERMAL NCO EXPOSURE RISK THROUGH TASKS OTHER THAN COMPOUNDING:

Compounding was the only end-user work task that was investigated in this study. Other tasks such as dry or wet sanding may pose some risk of NCO skin exposure given that in practice workers seldom wear gloves while performing them, even though both can involve considerable deposition of finely ground PU coating material upon the skin. In addition, given that water can amplify the rate of chemical absorption through an individual's skin, wet sanding may

potentially pose a greater risk of internal NCO exposure than compounding, if transfer to skin happens to occur. In one of the aforementioned studies which conducted skin wipe sampling for quantitative analysis immediately following various worker tasks, 8/10 samples collected after un-taping and 8/10 samples taken post wet sanding were found to have detectable levels of total NCO.[13] In that investigation wet sanding samples had the highest free isocyanate recovery of any of the non-spraying or non-mixing tasks evaluated yet even the geometric mean isocyanate level detected for un-taping was higher than compounding as well. Although the greatest potential for NCO skin exposure among auto body shop workers most likely occurs during direct paint-related procedures such as spray painting, the risk associated with wet sanding and the removal of masking tape following car surface paint applications warrants further exploration.

5D STUDY STRENGTHS AND LIMITATIONS:

Strengths of this study include on site conduct of the sampling in auto body shops, using actual workers, equipment, paints and procedures. Ten primers and clearcoats were tested in an attempt to sample a representative number of products. Standardized hand washing was implemented and the rubbing protocol was performed by auto body shop technicians who were not the painters who sprayed the coatings to be tested, so as to minimize the risk of dermal contamination by overspray from painting. Additionally, all field sampling was carried out by the same study personnel, using a set methodology with all sampling and scoring tests performed by a single investigator. Laboratory analyses were performed using validated and sensitive methods to detect individual isocyanate species per sample for total NCO calculation, plus field blanks, bulk and control samples were also collected to help rule out the presence of any cross-contamination (Figure 6). Furthermore, both surface quantitative wipe HPLC and qualitative

SWYPE™ analysis were employed and correlated with two separate methods to sample skin that included: tape-stripping and wipe sampling, which showed similar results (i.e. little or no detectable free NCO). Importantly, both pre- and post-compounding samples were taken to better assess NCO exposure risk through performance of the task.

Some study weaknesses exist which were mostly unavoidable. Of note, the number of samples below LOD limited statistical analyses. Nevertheless, falsely negative results could have occurred for several reasons. The recovery of NCO from the skin tape-strips or wipe samples could have been suboptimal, but the similar no or low detectable amounts with both methods suggests that recovery, even if not ideal, was adequate. All methods used to detect isocyanate species rely on free NCO, so rapid reaction of these groups with moisture or skin proteins could lead to falsely low levels of free NCO being detected in the skin tape-strip or wipe samples. Every effort was made to perform all sampling as soon as sprayed test panels were considered to be dry at t_0, as well as immediately after the rubbing protocol and compounding task, to minimize the possibility of false negative results. Nevertheless, limited study manpower and participant work obligations resulted in some panels continuing to dry while sampling occurred on others or waiting for workers to become available, so that the sampling times were delayed in some cases. In addition, the painters' determinations that parts were dry were partially subjective and somewhat variable, which nevertheless benefitted the realism of our investigation.

Adherence to a strict pre-sampling hand washing and drying protocol for all participants may also have resulted in excessive removal of natural layers of stratum corneum and oils from the hands, which may normally facilitate the transfer and absorption of NCO from surfaces to skin. Quite the opposite, the frictional forces placed on worker digits during the thorough hand washing protocol may have resulted in mild dermal abrasion and diminished barrier function

leading to an enhanced absorption prior to skin sampling and in turn a poorer recovery of NCO species. However, the aforementioned scenarios are merely speculative and if anything, reinforce the possibility that unbound surface NCO contamination and the potential transfer of these isocyanate groups to worker skin may in fact be higher in actual practice.

5E RECOMMENDATIONS AND FUTURE RESEARCH:

Given that isocyanate species found in the hardeners of both clearcoats and primers readily undergo exothermic reactions when in direct contact with active hydrogen atoms, intuitively one would expect ambient paint drying conditions with higher relative humidity to result in shorter curing times with less unbound NCO surface contamination in such settings. However, under the specific conditions as shown by the interaction model in Figure 2 for primers, ascending relative humidity is predicted to result in the presence of reduced total NCO on surfaces only to approximately 73 °F. The apparent inverse relationship observed for temperatures in excess of 73 °F is not obvious. Above this ambient temperature the opposite relationship is predicted whereby higher percentages of relative humidity correspond to increased surface unbound isocyanate contamination and perhaps greater risk of dermal exposure. Therefore, it may be advantageous for auto body shops to use air conditioning, particularly during the summer months in order to reduce ambient humidity and temperature conditions. Future research is needed to identify and better understand the factors which modify the rates of reaction and absorption of isocyanate species through human skin. An improved understanding of the extent to which human dermal and respiratory sensitization to isocyanate species occurs is also essential particularly within the automotive repair setting even though these questions are extremely challenging to address.

Finally, while attempts were made to sample representative aliphatic isocyanate coatings used in auto body shops and under typical work conditions, there are numerous different products in use under a range of working conditions that could impact the risk of skin exposure to free NCO, yet only a few tasks and products were sampled here. Despite these many caveats, the risk of isocyanate skin exposure to workers from car parts that appear to be dry most likely is relatively small. Our data also show that this risk could potentially be reduced even further by ensuring additional drying time before contact with the surfaces, especially those painted with clearcoat, as well as by ensuring worker PPE (i.e. gloves) utilization during tasks which involve any sort of tactile manipulation of potentially uncured NCO species even when these procedures may not directly involve spray painting.

5F CONCLUSION:

Although quantifiable unbound NCO groups were detected on the majority of surfaces which were recently painted with aliphatic isocyanate coatings and that appeared dry, the data presented here demonstrate limited transfer of free NCO from these surfaces to the skin of workers who handled such dried but perhaps incompletely cured test panels. Thus, risk of isocyanate skin exposure from direct skin contact with recently painted and dried car surfaces appears to be low under the circumstances evaluated.

The kinetics of NCO curing were influenced by several factors including coating type, drying method, hardener ratio, as well as the ambient temperature and humidity during application of the paints. Surfaces spray-painted and then compounded were frequently positive qualitatively for unbound NCO yet showed an incongruence comparing paired qualitative and quantitative surface samples, suggesting the possible generation of thermal NCO degradation

products, which were unidentified by the quantitative HPLC methodology. Such thermal degradation products if transferred to skin would have been difficult to detect with only the same HPLC methodology available for skin analysis. The risk of substantial skin exposure to isocyanate from touching dried surfaces and compounding was low under the conditions studied. Whether other shop tasks (e.g. wet sanding or un-taping) can result in the transfer of free NCO from auto body surfaces to human skin is an important area for future investigation.

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Tables.

Table 1. Description of participant characteristics (n=18)

Age (years)	
Mean ± SD (range)	30.5 ± 9.5 (21-47)
Gender (n, %)	
Male	18 (100%)
Race (n, %)	
Caucasian	15 (83%)
Hispanic	2 (11%)
African-American	1 (6%)
Education (n, %)	
Less than 12 years	1 (6%)
Graduated high school	12 (66%)
Technical/associate or some college	3 (17%)
College degree or more	2 (11%)
Smoking Status (n, %)	
Never smoked	6 (33%)
Ex-smoker	5 (28%)
Current smoker	7 (39%)
If smoked, pack years median (IQR, range)	7.5 (9.5, <0.1-35)
History of asthma (n, %)	
Onset after starting auto body work	0 (0%)
Childhood (onset <15 yrs. old)	4 (22%)
Current Asthma	0 (0%)
Occupational Exposure	
Duration in current auto body position (years) median (IQR, range)	4.0 (6.3, 0.3-28)
Job category (n, %)	
Painter	6 (33%)
Technician	12 (67%)
SD = Standard deviation. IQR = Interquartile range.	

Table 2. Description of auto body shop characteristics (n=5)

	mean	**range**
Age of shop (years)	39.25	16-57
Size of shop (ft^2)	9,500	7,000-33,000
Cars painted per month	62	40-90
Annual income ($1,000)	1,080	450-1,600
Non-office employees	7	3-9
Number of booths	1.7	1-3

Table 3. Overview of collected study samples

				Surface		***Skin***			
Sampling times	*Number of shops*	*Number of painters*	*Number of workers*	*Number of qualitative wipe samples*	*Number of quantitative wipe samples*	*Number of tape-strip samples (control)*	*Number of tape-strip samples*	*Number of quantitative wipes (control)*	*Number of quantitative wipes*
Initial time point (t_0)									
Clearcoat	5	5	13	12	28	9	44	4	16
Primer	4	4	8	8	10	7	20	0	0
Time course (t_0-t_3)									
t_0 (≈0 hrs post-dry)	2	2	4	4	4*	4*	8*		
t_1 (≈1.5 hrs post-dry)	2	2	4	0	4	0	8		
t_2 (≈3 hours post-dry)	2	2	4	0	4	0	8		
t_3 (≈24 hrs post-dry)	2	2	4	4	4	4	8		
Task-related									
Pre-compounding	3	3	6	6	6*	0**	12*		
Post-compounding	3	3	6	6	6	5	12		

*also counted in initial time point (t_0) samples
**identical to/performed as initial time point (t_0) clearcoat controls

Table 4. Aliphatic isocyanate (μg NCO cm^{-2}) detected on surfaces and skin (time t_0)

	Surface										***Skin***			
Coating Type	# QN wipes	pHDI		pIPDI		HDI monomer		Total NCO			# Tape-strips	# >LOD(%)	# QN Skin Wipes	# >LOD (%)
	n	GM (GSD)	% >LOD	GM (GSD)	% >LOD	GM (GSD)	% >LOD	GM (GSD)	% >LOD	Range				
Clearcoat	28	0.038 (38.7)	75	0.393 (3.2)	57	0.003 (7.3)	82	0.139 (18.4)	86	<0.001-3.268	44	2 (5%)	16	2 (13%)
Primer	10	0.043 (19.7)	80	0.158 (1.3)	40	0.001 (6.1)	60	0.063 (17.1)	80	<0.001-0.439	20	3 (15%)	0	0
All Samples	38	0.039 (31.5)	76	0.327 (3.0)	53	0.002 (7.4)	76	0.113 (17.7)	84	<0.001-3.268	64	5 (8%)	16	2 (13%)

QN = Quantitative wipe samples
HDI = Monomeric hexamethylene diisocyanate
pHDI = Polymeric hexamethylene diisocyanate
pIPDI = Polymeric isophorone diisocyanate
Total NCO = calculated by individual sample as the sum of HDI, pHDI and pIPDI species
GM = Geometric mean
GSD = Geometric standard deviation
½ Limit of detection (LOD) was used for pHDI in calculations if the individual sample value was ≤ LOD (0.005 μg NCO)

Table 5. Summary of isocyanate (µg NCO) content among all positive skin samples (n=7)

Sample Type	Coating type	HDI monomer	Selected pHDI species			pIPDI	Total NCO (per sample)	Total NCO (µg NCO cm^{-2})
			dione	biuret	isocyanurate			
Tape-strip n=5	Pr	ND	ND	ND	0.003	0.004	0.007	0.001
	Pr	ND	ND	ND	0.011	ND	0.011	0.002
	CC	ND	ND	ND	0.003	ND	0.008*	0.002
	CC	0.078	0.015	0.041	3.254	ND	4.394*	0.879
	CC	0.062	0.030	0.026	2.736	ND	3.942*	0.788
Wipe n=2	CC	ND	0.008	0.008	0.080	0.027	0.218*	0.032
	CC	ND	ND	ND	0.005	0.012	0.017	0.002

CC = Clearcoat Pr = Primer ND = Non-detectable (≤ LOD)

HDI: LOD 0.001-0.004 µg NCO depending on sample matrix
Dione, biuret and isocyanurate: LOD 0.001-0.004 µg NCO depending on sample matrix
pIPDI: LOD <0.001 µg NCO

*Sample also contains other pHDI species not shown
Also see notes to Table 3

Table 6. Multivariate regression model parameter estimates for factors predicting ln(µg total NCO) surface contamination at time t_0

Parameter	***Estimate***	***Standard Error***	***t Value***	***Pr > |t|***
Baked surface	6.41729257	0.58899020	10.90	<0.0001
Clearcoated surface	-5.19640413	2.05352456	-2.53	0.0169
Temperature during painting (°F)	-0.75780993	0.09450198	-8.02	<0.0001
Humidity during painting (%)	-0.97851829	0.17804549	-5.50	<0.0001
Coating hardener ratio (% isocyanate)	-30.55101698	8.74220243	-3.49	0.0015
Coating type (CC=1) and hardener ratio interaction	24.93510917	10.63392050	2.34	0.0258
Painting humidity and temperature interaction	0.01329424	0.00226074	5.88	<0.0001

Table 7. Aliphatic isocyanate (μg NCO cm^{-2}) detected on surfaces and skin over time (t_0-t_3)

	Surface										***Skin***	
		pHDI		pIPDI		HDI monomer		Total NCO			# Tape-strip Samples (n)	Samples with Total NCO >LOD n (%)
	n	GM (GSD)	% >LOD	GM (GSD)	% >LOD	GM (GSD)	% >LOD	GM (GSD)	% >LOD	Range		
t_0 (≈0 hours post-dry)	4	0.156 (1.2)	100	0.181 (1.3)	100	0.001 (11.1)	50	0.339 (1.2)	100	0.263-0.411	8	1 (13%)
t_1 (≈1.5 hours post-dry)	4	0.070 (2.0)	100	0.101 (1.9)	100	0.002 (1.8)	100	0.174 (1.9)	100	0.080-0.306	8	0 (0%)
t_2 (≈3 hours post-dry)	4	0.024 (5.9)	100	0.071 (2.4)	100	0.001 (2.8)	100	0.104 (2.7)	100	0.026-0.217	8	0 (0%)
t_3 (≈24 hours post-dry)	4	0.005 (2.3)	100	0.032 (1.7)	100	0.001 (2.7)	75	0.040 (1.6)	100	0.020-0.056	8	0 (0%)
See notes to Table 3												

Table 8. Aliphatic isocyanate (µg NCO cm^{-2}) detected on surfaces and skin before and after compounding task

	Surface										***Skin***	
	# QN wipes	pHDI		pIPDI		HDI monomer		Total NCO			# Tape-strip Samples (n)	Samples with Total NCO >LOD n (%)
	n	GM (GSD)	% >LOD	GM (GSD)	% >LOD	GM (GSD)	% >LOD	GM (GSD)	% >LOD	Range		
Pre-compounding	6	0.036 (19.8)	83	0.141 (1.5)	33	0.003 (4.0)	83	0.176 (2.2)	100	0.077-0.508	12	0 (0%)
Post-compounding	6	0.005 (42.3)	50	0.075 (1.4)	33	0.001 (6.9)	50	0.042 (11.6)	83	<0.001-0.217	12	1 (8%)
See notes to Table 3												

Figures.

Figure 1. Effect of coating type and hardener ratio on ln(total NCO) with panel air dried after coating application at 72 °F and 50% humidity

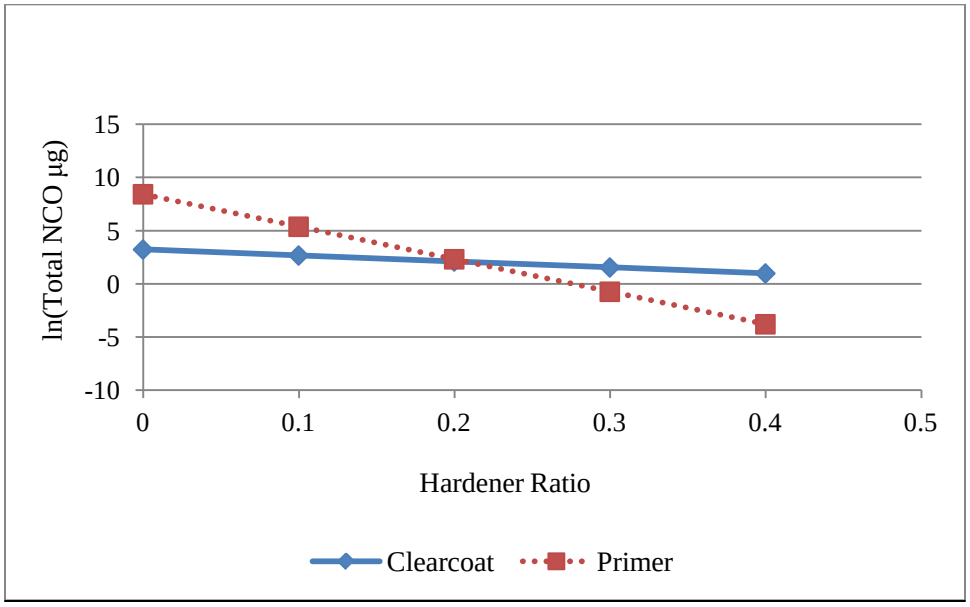


Figure 2. Effect of humidity and temperature on ln(total NCO) for panel coated with primer of 20% hardener ratio and air dried

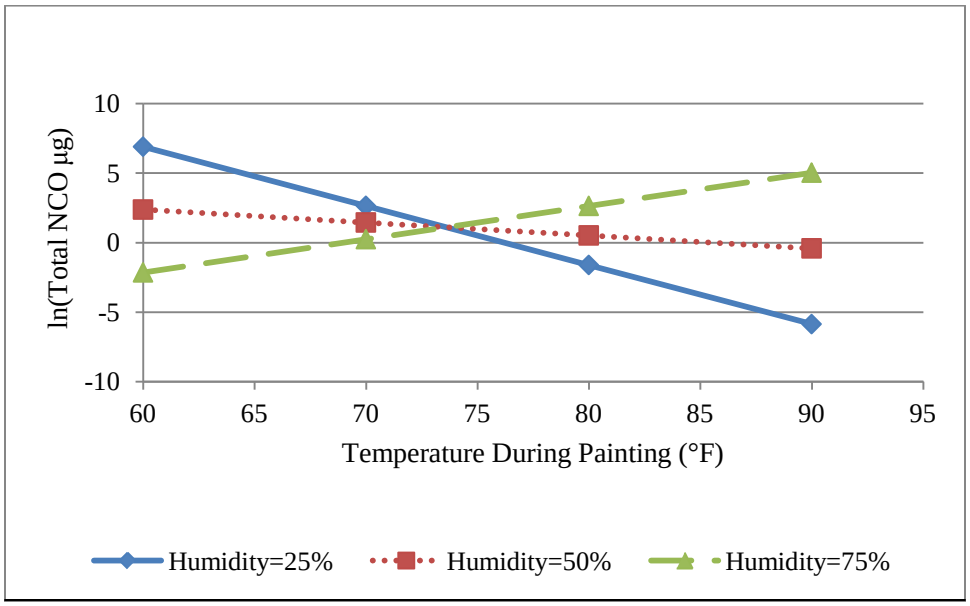


Figure 3. Decay curves of recovered unbound total NCO for tested primer and clearcoat over the 24 hours post dry time

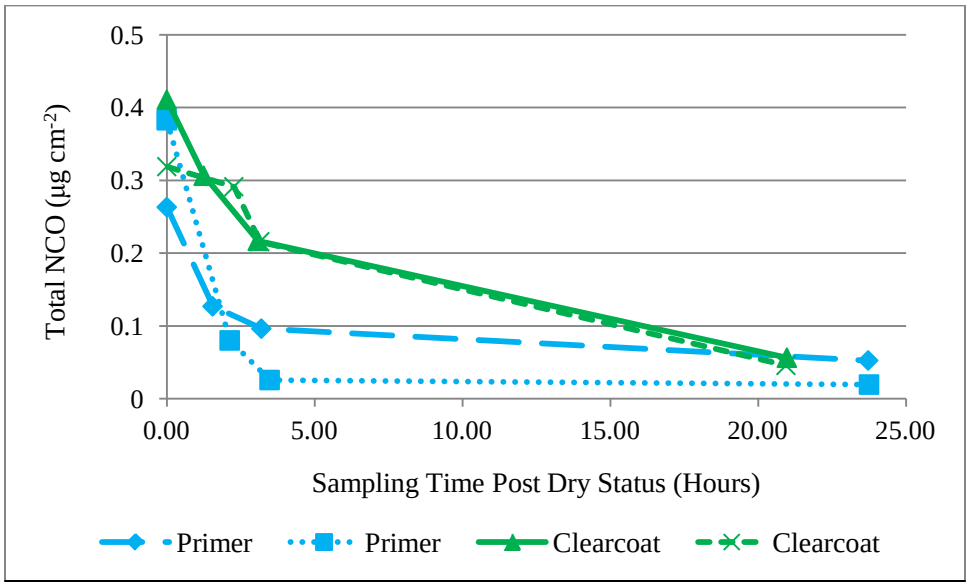


Figure 4. Qualitative surface sampling results by individual test panel before and after compounding

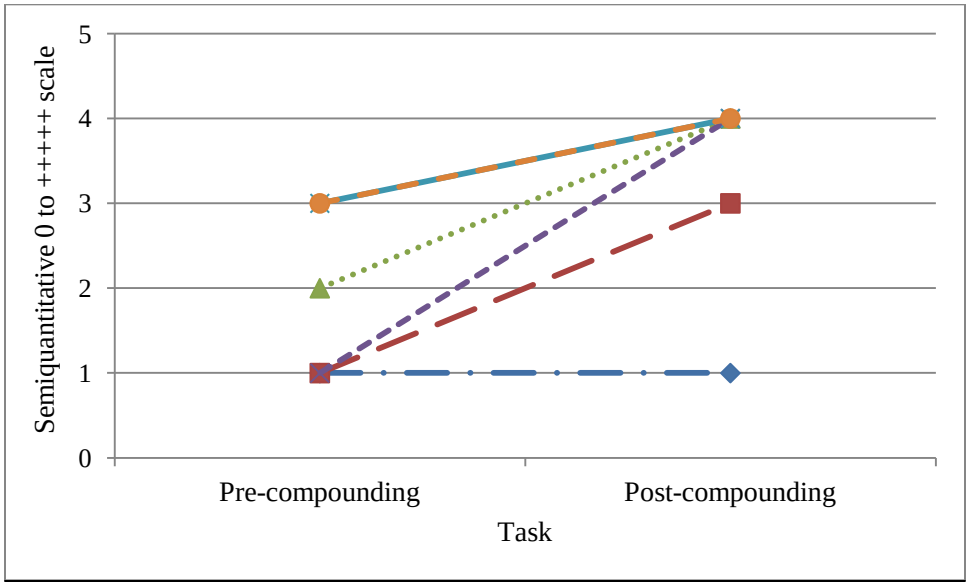


Figure 5. Quantitative surface sampling results by individual test panel before and after compounding

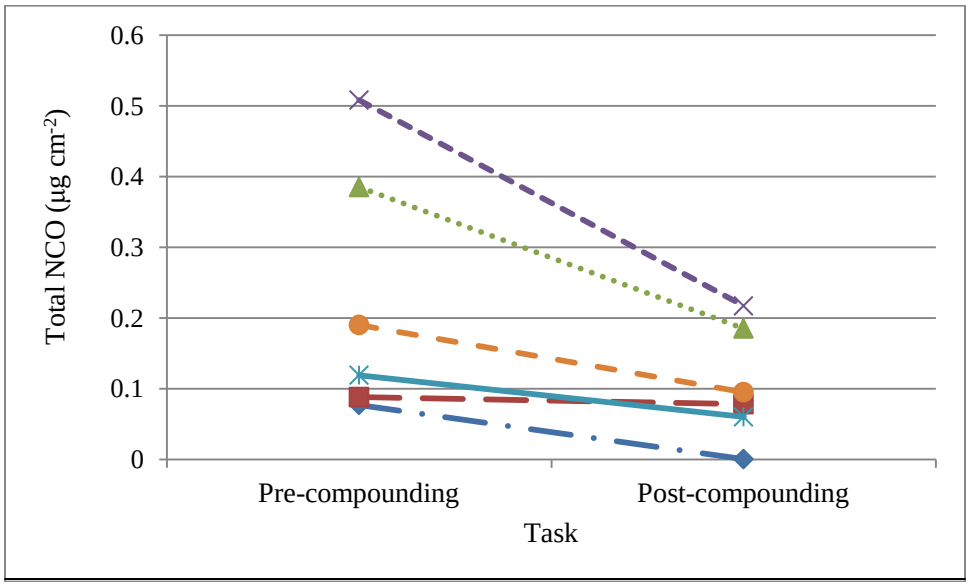
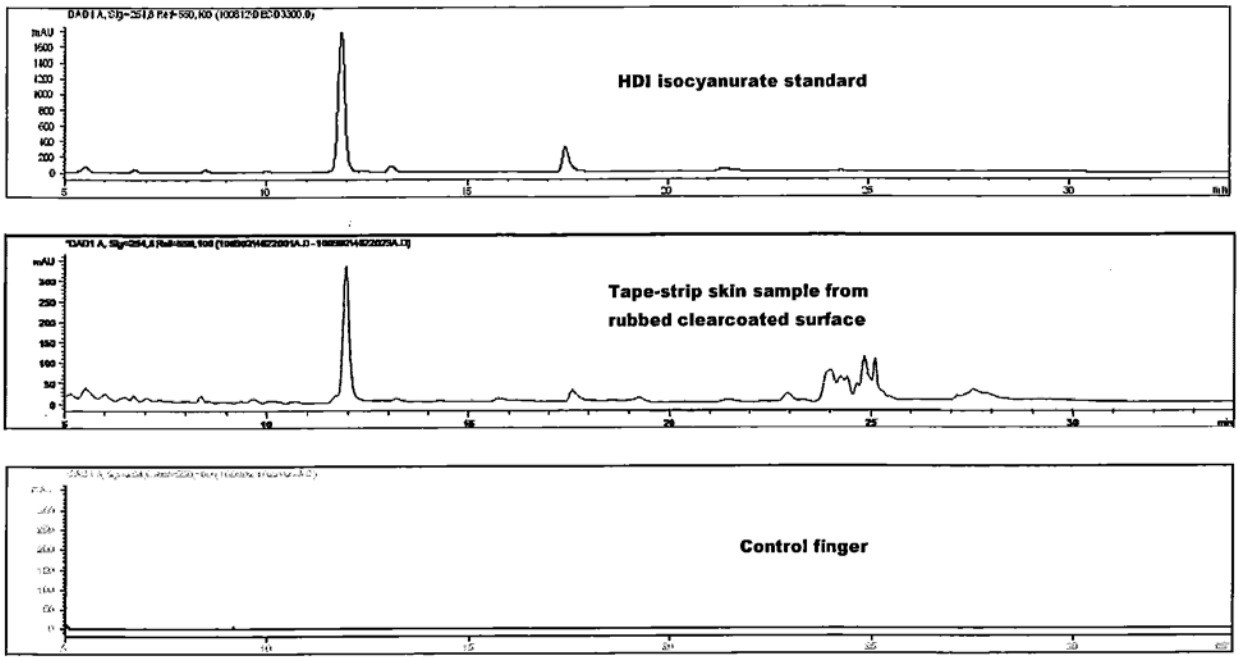


Figure 6. Selected study sample chromatograms



The chromatogram for a tape-strip skin sample (middle figure) is shown whose monophasic peak at a retention time of approximately 12 minutes corresponds to the standard for HDI isocyanurate (a pHDI species) (upper figure). The cluster of moderate signal intensity peaks around 24 minutes are characteristic of pIPDI whose standard is not shown here. As expected, the corresponding control tape-strip sample chromatogram (lower figure) demonstrates no detectable signal, which is un-suggestive of cross-contamination.

Appendix A. Participant Survey Instrument

ALIPHATIC ISOCYANATE SKIN TRANSFERABILITY
IN AUTOMOTIVE REFINISHING

PPT ID #_____
Shop _____
Date _____

Name: _____ _____ _____ Date of Birth: _____
Last *First* *Middle* *(Mo/Day/Year)*

Home Address: _____
Street *City* *State* *Zip*

Telephone: _____ _____ _____
Daytime *Evening* *Cell*

Gender: Male Female Ethnicity: Do you have Hispanic background? Yes No

Race: What race do you consider yourself to be?
Caucasian Spanish/Hispanic Native American or Eskimo
African American Asian Other _____

Last Education Level Completed/Highest Degree: High school College/Other Post Graduate

Smoking: 1. Have you ever smoked cigarettes? Yes No
1a. If yes, how many years have you smoked? _____ years
1b. Do you currently smoke cigarettes (as of 1 month ago)? Yes No
1c. If no, how many years since you quit? _____ years
1d. During the time you smoked, how many cigarettes did you smoke per day on average? _____

Allergies: 2. Do you have allergies such as hay fever (runny nose, itchy eyes) during certain seasons (like pollen season)? Yes No

Asthma: 3. Have you ever had asthma or been told you have asthma? Yes No Don't know (DK)
3a. If yes, about what age did the asthma start? _____
3b. Were you working then? No Yes: 3c. where? _____
3d. Was your asthma confirmed by a doctor? Yes No
3e. Do you still have asthma? Yes No

Asthma Symptoms

Do you currently have:	**a.**	**b. work-related?**	**c. after work/ at night?**
4. wheezing or whistling in your breathing	Yes No	Yes No	Yes No
5. attacks of shortness of breath	Yes No	Yes No	Yes No
6. chest tightness	Yes No	Yes No	Yes No
7. cough attacks	Yes No	Yes No	Yes No
8. nasal or eye symptoms (sneezing, runny/itchy nose, itchy watery eyes)	Yes No	Yes No	Yes No
9. skin rash, possibly work-related	Yes No	Yes No	Yes No

10. Have you ever noticed that a particular task or product causes any of these symptoms? Yes No
10a. If yes, what task or product? _____

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Medications/Inhalers : 11. Do you take any medications regularly, including inhalers and non-prescription medicine except for vitamins? Yes: list below No

Medication	Dose	Frequency
11a.	11a1.	11a2.
11b.	11b1.	11b2.
11c.	11c1.	11c2.
11d.	11d1.	11d2.
11e.	11e1.	11e2.

Current job: 12. Job title: _____

12a. How long have you been working at this job? _____ years _____ months

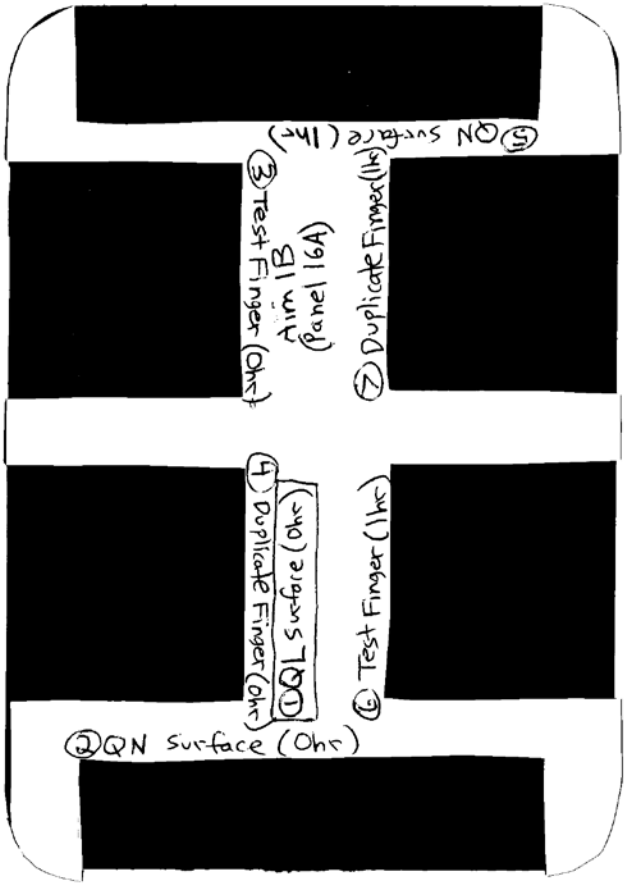
Tasks: Please describe all tasks you do:

		1. Iso-containing paint?	2. PPE used
13a. painting	Yes No	Yes No	
13b. priming	Yes No	Yes No	
13c. sanding	Yes No	Yes No	
13d. compounding	Yes No	Yes No	
13e. unmasking	Yes No	Yes No	

14. This study will involve touching dried isocyanate-containing paints. Please tell us if you have never touched dried isocyanate-containing paint without gloves. Yes, have touched No, never touched

15. Have you ever noticed any isocyanate-containing paints or coatings on your skin (hands, face, under clothes)?
Yes No

Appendix B. Selected Test Panel Template and Corresponding Data Collection Form



Selected study test panel with overlying template. (Image is not to scale).

Aim 1B

Date(s):

Shop Name: _____ Shop ID #: _____ Sampled By: _____ Test Panel ID#s: _____

Painter Name/ID#: _____ Worker 1 Name/ID#: _____ Worker 2 Name/ID#: _____

Paint Product Component Information Bulk ID ____

Coating Type: Primer/ Clear coat	Product Brand Name and #	Hardener Brand & #	Reducer Brand & Number	Ratio of Product: Hardener : Reducer	Gallons Used Per Month
Pr CC					

Number of Coats Applied: _____ Booth Type _____ Drying Method: _____

Drying Time Between Application and Sampling (t_0) (min.): _____ Time Considered Dry _____

Indoor Meteorology	At Time of Painting	During Drying	During Sampling
Temperature (°F)			
Relative Humidity (%)			

Surface Wipe Sampling

	Qualitative	Result (- to +++++)	Sample ID #
Time ___	Panel # __a		
Time ___	Panel # __b		

Quantitative

Panel # ___ Time ___ Panel # ___ Time ___ Panel # ___ Time ___ Panel # ___ Time ___

Place 0 Hours Label Sticker Here	Place 1 Hour Label Sticker Here	Place 3 Hours Label Sticker Here	Place 24 Hours Label Sticker Here
Place 0 Hours Label Sticker Here	Place 1 Hour Label Sticker Here	Place 3 Hours Label Sticker Here	Place 24 Hours Label Sticker Here

Tape-Strip Sampling

Worker 1

0 Hours Test Area + Sample ID # Strip 1 & 2		1 Hour Test Area + Sample ID # Strip 1 & 2		3 Hours Test Area + Sample ID # Strip 1 & 2		24 Hours Test Area + Sample ID # Strip 1 & 2	
Control Hand: R or L Finger: T I M R or P	___ -3A0	X		X		Control Hand: R or L Finger: T I M R or P	___ -3A24
Test Hand: R or L Finger: T I M R or P	___ -3B0	Test Hand: R or L Finger: T I M R or P	___ -3B1	Test Hand: R or L Finger: T I M R or P	___ -3B3	Test Hand: R or L Finger: T I M R or P	___ -3B24
Duplicate Test Hand: R or L Finger: T I M R or P	___ -3C0	Duplicate Test Hand: R or L Finger: T I M R or P	___ -3C1	Duplicate Test Hand: R or L Finger: T I M R or P	___ -3C3	Duplicate Test Hand: R or L Finger: T I M R or P	___ -3C24
Panel # ___ Time/Temp/Hum: ___ ___ ___		Panel # ___ Time/Temp/Hum: ___ ___ ___		Panel # ___ Time/Temp/Hum: ___ ___ ___		Panel # ___ Time/Temp/Hum: ___ ___ ___	

Worker 2

0 Hours Test Area + Sample ID # Strip 1 & 2		1 Hour Test Area + Sample ID # Strip 1 & 2		3 Hours Test Area + Sample ID # Strip 1 & 2		24 Hours Test Area + Sample ID # Strip 1 & 2	
Control Hand: R or L Finger: T I M R or P	___ -4A0	X		X		Control Hand: R or L Finger: T I M R or P	___ -4A24
Test Hand: R or L Finger: T I M R or P	___ -4B0	Test Hand: R or L Finger: T I M R or P	___ -4B1	Test Hand: R or L Finger: T I M R or P	___ -4B3	Test Hand: R or L Finger: T I M R or P	___ -4B24
Duplicate Test Hand: R or L Finger: T I M R or P	___ -4C0	Duplicate Test Hand: R or L Finger: T I M R or P	___ -4C1	Duplicate Test Hand: R or L Finger: T I M R or P	___ -4C3	Duplicate Test Hand: R or L Finger: T I M R or P	___ -4C24
Panel # ___ Time/Temp/Hum: ___ ___ ___		Panel # ___ Time/Temp/Hum: ___ ___ ___		Panel # ___ Time/Temp/Hum: ___ ___ ___		Panel # ___ Time/Temp/Hum: ___ ___ ___	