An FTIR investigation of isocyanate skin absorption using *in vitro* guinea pig skin†

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Isocyanates may cause contact dermatitis, sensitization and asthma. Dermal exposure to aliphatic and aromatic isocyanates can occur in various exposure settings. The fate of isocyanates on skin is an important unanswered question. Do they react and bind to the outer layer of skin or do they penetrate through the epidermis as unreacted compounds? Knowing the kinetics of these processes is important in developing dermal exposure sampling or decontamination strategies, as well as understanding potential health implications such exposure may have. In this paper the residence time of model isocyanates on hairless guinea pig skin was investigated *in vitro* using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectrometry. Model isocyanates tested were octyl isocyanate, polymeric hexamethylene diisocyanate isocyanurate (pHDI), polymeric isophorone diisocyanate isocyanurate (pIPDI) and methylenediphenyl diisocyanate (MDI). Isocyanates in ethyl acetate (30 μL) were spiked directly on the skin to give 0.2–1.8 μmol NCO cm^{-2} (NCO = –N═C═O), and absorbance of the isocyanate group and other chemical groups of the molecule were monitored over time. The ATR-FTIR findings showed that polymeric isocyanates pHDI and pIPDI may remain on the skin as unreacted species for many hours, with only 15–20% of the total isocyanate group disappearing in one hour, while smaller compounds octyl isocyanate and MDI rapidly disappear from the skin surface (80 + % in 30 min). Isocyanates most likely leave the skin surface by diffusion predominantly, with minimal reaction with surface proteins. The significance of these findings and their implications for dermal exposure sampling and isocyanate skin decontamination are discussed.

Introduction

Isocyanates are a group of reactive chemicals with the functional group N═C═O (abbreviated as NCO) that are extensively used in the manufacture of various commercial products, such as coatings and polyurethane plastics. Exposure to isocyanates can cause contact dermatitis, skin, eye, and respiratory tract irritation, sensitization and asthma, and less commonly hypersensitivity pneumonitis.[1] Asthma remains the isocyanate-related adverse health effect of primary concern. Skin exposure to isocyanates is implicated directly in developing skin irritation and/or contact dermatitis.[2–4] Animal studies demonstrate that dermal exposure to isocyanates is an effective route to induce sensitization and that subsequent inhalation challenge can result in asthma.[5,6] For example, for toluene diisocyanate (TDI)[6] and diphenylmethane diisocyanate (MDI)[5] sensitization in animals was induced by dermal contact alone, with subsequent inhalation exposure resulting in a respiratory response. Two recent murine models, one for HDI[7] and one for TDI[8] have used skin sensitization followed by inhalation challenge to create an asthmatic response). In light of the animal data it is likely that skin may be an exposure route and risk factor for sensitization in humans.

Data demonstrating human isocyanate skin exposure can lead to sensitization is much more limited. A study among workers exposed to MDI-containing resins in a facility designed for minimal airborne exposures found a correlation between the high skin and clothing exposure potential to liquid MDI monomer and prepolymer and new asthma-like respiratory symptoms.[9]

Dermal exposure to isocyanates in the workplace is common.[10,11] However, little is known about the fate of the isocyanate following skin contact. Does it bind to the skin? Does it penetrate through the skin unreacted? Is one of these processes dominant and what are the kinetics? Understanding these processes should help in developing better skin decontamination procedures, dermal sampling techniques, and improved control strategies for isocyanates.

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Isocyanates react with nucleophiles of the general form H–X–R′, such as amines R′–NH_2, alcohols R′–OH, water H_2O, carboxylic acids R′–COOH, and thiols R′–SH in a nucleophilic addition reaction as depicted in eqn (1):[12]

$$R–N{=}C{=}O + H–X–R' \rightarrow R–NH–CO–X–R' \qquad (1)$$

The outermost layer of the epidermis of the human skin, the stratum corneum (SC), is composed on a dry weight basis of ~75–80% protein (mostly as α-keratin, located within the dead keratinocytes), 10–15% lipids, and the remainder 5–10% unidentified components.[13] The lipid content, organized in multiple bilayers surrounding the keratinocytes, is rich in ceramides, free fatty acids, and cholesterol. Smaller amounts of phospholipids and free amino acids such as serine, glycine, and histidine are also found in the SC.[13] The SC, therefore, is rich in OH (ceramides, cholesterol, water, carboxylic acids, *etc.*), NH groups (ceramides, amino acids, enzymes/proteins), and SH (cysteine residues in keratin), which can spontaneously react with NCO groups, without catalysts. Water generated during sweating can also react with isocyanates.

The high reactivity of isocyanates towards the NH and OH groups in the SC raises the possibility that isocyanates may react quickly with, and bind to, the SC. The metabolic fate and kinetics of isocyanate reaction with skin remain unclear.

Skin wiping has been used in occupational settings both as a sampling technique and for decontamination. Chemical reactivity of isocyanates, however, complicates interpretation of results obtained from skin wiping or decontamination studies. Variable isocyanate recoveries may result due to isocyanate binding to the outermost layer of the skin, permeation through the skin, incomplete removal due to skin porosity, or chemical destruction of isocyanate groups by the solvent/decontaminant. The relative contributions of such processes are largely unknown and are difficult to investigate with wiping techniques. Therefore, direct measurements on the skin, by methods other than wiping, are required. In a previous study[14] we employed a combination of techniques, including investigation of reaction kinetics of various decontaminants with model isocyanates and physical removal efficiency of isocyanate spikes on aluminum foil (a material with very low porosity), to distinguish between chemical destruction and physical removal of isocyanates. This design, however, could not address the unique issues related to the skin.

ATR-FTIR is a surface analytical technique which has been successfully used for a variety of *in vitro* and *in vivo* applications, including measuring skin exposure to pesticides[15] and characterizing percutaneous absorption of other chemicals.[16–19] The depth of penetration of the IR beam into the SC, the outermost and least permeable layer of the human skin, depends on optical parameters such as the ATR crystal material, refractive index of the skin, incident beam angle and wavelength, and is in the order of 1 μm. Given that a typical layer of corneocytes is 0.2–1.5 μm thick,[13] only the top 1–5 cell layers deep in the SC are probed.[20] The SC is a non-uniform membrane, typically comprised of 10–15 cell layers of corneocytes and is around 10–15 μm thick when dry.[13,16] The ATR-FTIR measures absorption of the IR beam by chemical groups in the molecule, such as the N=C=O bond of isocyanates, and the absorption intensity is proportional to the concentration of the chemical.

The main objectives of this study were to (i) investigate the feasibility of attenuated total reflectance-Fourier transform infrared spectrometry (ATR-FTIR) as a dermal sampling technique for isocyanates; and (ii) to gain insight into the fate of the isocyanate group/molecule on the skin and the kinetics of chemical reaction with the SC. Model isocyanates were spiked *in vitro* on previously frozen hairless guinea pig skin and the isocyanate was monitored with ATR-FTIR.

Materials and methods

Instrumentation

The instrument used for this work was a Nicolet 4700 FTIR Spectrometer (Thermo Electron Co., Madison, WI), equipped with a Smart Orbit single bounce ATR accessory with a diamond crystal (~3 mm diameter). The data were acquired and processed with the Omnic 7.1 software. Thirty-two scans were collected over 38 s for each run at a resolution of 4 cm^{-1} with apodization set to 'Happ-Genzel', phase correction to 'Mertz' and zero filling to 'none'. Skin background spectrum was used for background subtraction.

The hairless guinea pig (HL-GP) skin

Previously frozen hairless guinea pig skin, ~1 mm thick, was obtained from the National Institute for Occupational Safety and Health (NIOSH) and kept in airtight plastic freezer bags at −18 °C. Prior to conducting the experiments, the skin was thawed using plastic bags filled with warm water and slowly warmed to room temperature (22–24 °C). The skin was visually inspected for damage, such as bruises and cuts. The skin was then cut into small ~3 × 3 cm square pieces as needed for further experimentation. Since moisture is known to be an important determinant of skin permeability,[13] the subcutaneous side of the skin (facing up during measurements) was covered with aluminum foil to prevent dryness and minimize evaporative water loss. This did not interfere with experimental measurements. Skin washing or clean up to remove interfering lipids near the surface was not performed, as this might interfere with the barrier properties of the skin, an important determinant for these experiments. Only the ventral skin from two animals was used.

Model isocyanates

Four model isocyanates were tested. They were: octyl isocyanate 97% purity and MDI 98% purity (Aldrich Chemical Co., Milwaukee, WI), polymeric HDI isocyanurate (pHDI) and polymeric IPDI isocyanurate (pIPDI) (respectively, Desmodur N3300 and Desmodur Z4470, both from Bayer Co., Pittsburgh, PA). Desmodur N3300 (pHDI), marketed as a 100% liquid, contained ~22% NCO by weight. Desmodur Z4470 (pIPDI), marketed as a 70% mixture in 30% ethyl acetate, contained ~12% NCO by weight. Polymeric HDI and pIPDI are complex mixtures, of which the trimer isocyanurate is usually the major component. For pHDI the isocyanurate constitutes about two-thirds of the total NCO group, whereas for pIPDI the exact contribution is unknown. However, higher

polymers of both pHDI and pIPDI also contain isocyanurate structures and their total NCO contribution is substantially higher. Polymeric HDI and pIPDI are non-volatile at normal temperature, whereas the volatility of MDI is very low. Octyl isocyanate—a volatile monoisocyanate—was chosen for evaluation due to its simplicity in monitoring and interpreting the fate of a single NCO group, especially given our concerns of isocyanate group chemical reaction with the skin. Octyl isocyanate, would thus serve as a surrogate for low molecular weight diisocyanates, such as HDI (6 C chain) and uretidinedione (2 HDI molecules). The other three were chosen due to their commercial importance.

Skin application

Thirty microlitres (μL) of model isocyanates diluted in ethyl acetate were spiked directly on the skin over a circle area of 5 cm^2. Ethyl acetate was chosen because it is a common solvent for isocyanates; it evaporates quickly, it is non-reactive towards isocyanates, and has low toxicity. Four to five replicates per isocyanate type were generated at an average NCO concentration (μmol NCO cm^{-2}) of 1.84 for octyl isocyanate, 0.31 for pHDI, 0.27 for pIPDI, and 0.21 for MDI. This is an average NCO concentration calculated as total NCO load/surface area, but due to inhomogeneities, the real concentration on the skin surface in direct contact with the ATR crystal, as measured by the NCO peak height at time zero, typically differed by a factor of 2–3 between replicates. The whole experiment was randomized with regard to isocyanate type, concentration and skin section used. Replicates were randomly spread throughout the data collection period, separated by data analysis and other uses of the FTIR. After the solvent had completely evaporated (within about 15 s) the skin was pressed tightly against the crystal, and IR spectra were acquired every minute during the first 10 min, every 2 min for the next 20 min, and every 5 min afterwards for a period typically up to one hour. Exploratory individual measurements at substantially higher concentrations for longer time periods were also performed for MDI (2.1 μmol NCO cm^{-2}) and pHDI (12.4 μmol NCO cm^{-2}). Care was exercised to maintain reproducible conditions from run to run, such as by applying a constant pressure on the skin and preventing skin dryness.

FTIR spectral characteristics

The NCO group has a characteristic strong asymmetric stretching vibration around ~2270 cm^{-1} for all model isocyanates, except for pIPDI which absorbs at 2254 cm^{-1}. This absorbance is very useful for the identification and quantification of the NCO group. The unreactive carbonyl group (C═O) of the isocyanurate structure has a strong stretching vibration around 1680 cm^{-1}. Fig. 1 lists the chemical structures of isocyanates and their most prominent IR absorption bands used for quantification in this study.

The skin is expected to have strong absorption bands in the region 3400–2800 cm^{-1} primarily due to the strong OH stretch, around 1650 cm^{-1} due to the amide I band (carbonyl of the amide bond stretch), 1545 cm^{-1} due to the amide II band (NH deformation) of proteins and ~1740 cm^{-1} from the carbonyl stretching of fatty acids. The ~2270 cm^{-1} band of the NCO group occurs in a region of little interferences from other functional groups. Other bands occur in regions of strong skin absorption, and their quantitation requires either elimination of such interfering bands through skin pre-washing or skin background subtraction. Because pre-washing was undesirable, the background subtraction method was applied when necessary.

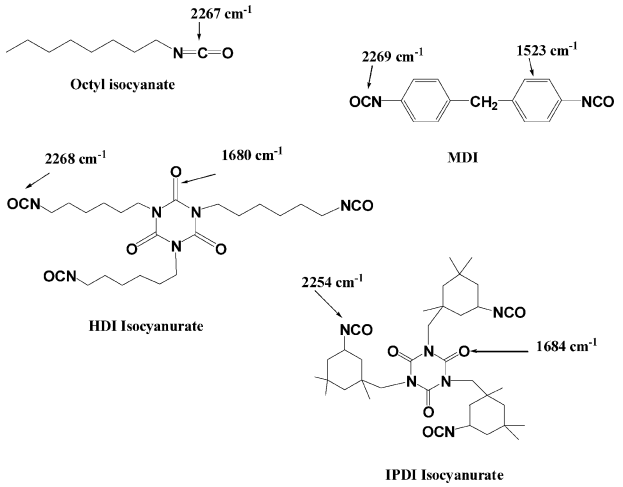


Fig. 1 Chemical structures of model isocyanates used in this study and their major infrared bands used for quantification purposes. The NCO groups for octyl isocyanate, MDI and pHDI absorb at ~2270 cm^{-1}, whereas for pIPDI at 2254 cm^{-1}. Note that while octyl isocyanate and MDI are pure products, pHDI (polymeric HDI isocyanurate) and pIPDI (polymeric IPDI isocyanurate) are mixtures, for which the structures shown represent the major component.

Results

FTIR spectra

A typical FTIR spectrum of pHDI isocyanurate and the HLGP skin are shown in Fig. 2. Small variations in the degree of

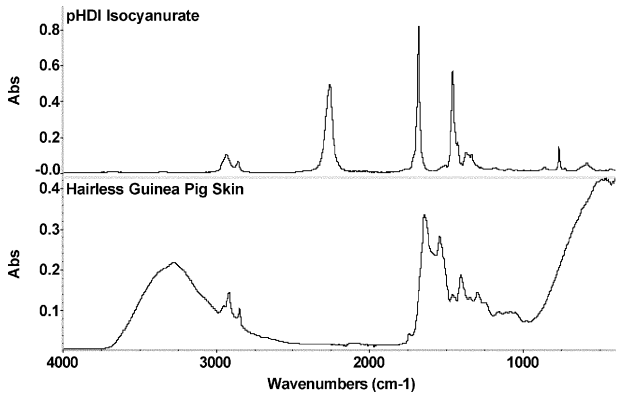


Fig. 2 A typical FTIR spectrum of pHDI isocyanurate (Bayer Desmodur N3300) (top) and hairless guinea pig skin (bottom). The asymmetric stretching vibration of the N═C═O group at ~2270 cm^{-1} of the pHDI spectrum is free of interferences from the skin matrix and enables direct monitoring of the NCO groups. The 1683 cm^{-1} band is due to the carbonyl C═O of the isocyanurate ring. The FTIR spectrum of the hairless guinea pig skin is very similar to the spectrum of human skin published in the literature.

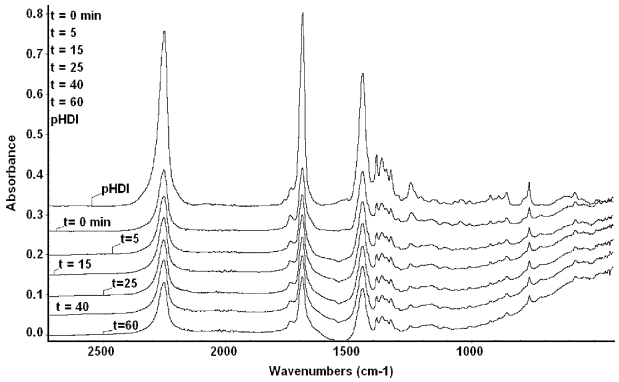


Fig. 3 FTIR kinetic data for pHDI (0.31 μmol NCO cm^{-2}) on the hairless guinea pig skin after skin background subtraction. Absorbance decreases by 37% from $t = 0$ to $t = 60$ min. The spectrum of pure pHDI (top) is provided for easy comparison.

hydration (as measured by the broad OH stretching frequency around 3300 cm^{-1}) and lipid content (as measured by the intense absorption at ~1740 cm^{-1}) were noticed. The lipid content and hydration level generally vary with the skin site and between skin types.

The NCO absorbance band of pHDI (Fig. 2) at ~2270 cm^{-1} is free of interferences and could be measured accurately without any spectral manipulation. The peaks at ~1680 or 1458 cm^{-1} could be measured with accuracy only after the skin blank was subtracted from the original spectrum. This is illustrated in Fig. 3 for the case of pHDI absorption during the first 60 min. During this time there was a reduction of 37% in the intensity of the NCO absorption band and no major spectral changes were observed.

Kinetics of absorption of isocyanates into SC

The change in the absorbance intensity for the NCO group of four model isocyanates from 0 to 60 min after each isocyanate is applied is presented in Fig. 4 (octyl isocyanate and pHDI), and Fig. 5 (MDI and pIPDI). The lines connect the mean

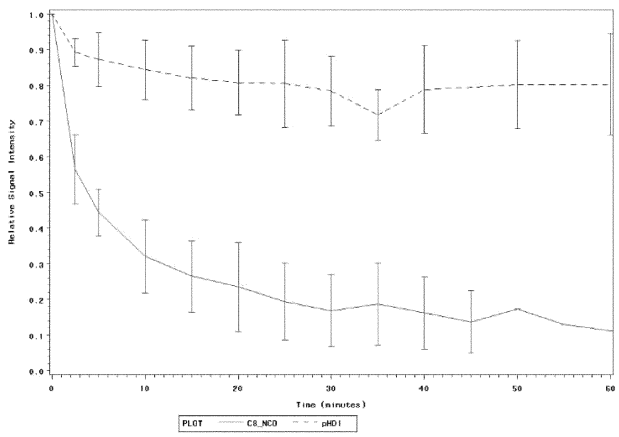


Fig. 4 Average change in the absorbance intensity of the N═C═O group at ~2270 cm^{-1} for octyl isocyanate (C8_NCO, 1.84 μmol NCO cm^{-2}, $n = 5$) and pHDI (0.31 μmol NCO cm^{-2}, $n = 4$). The line connects mean values of replicates and is not a fitted line. Error bars represent ±1 standard deviation from the mean.

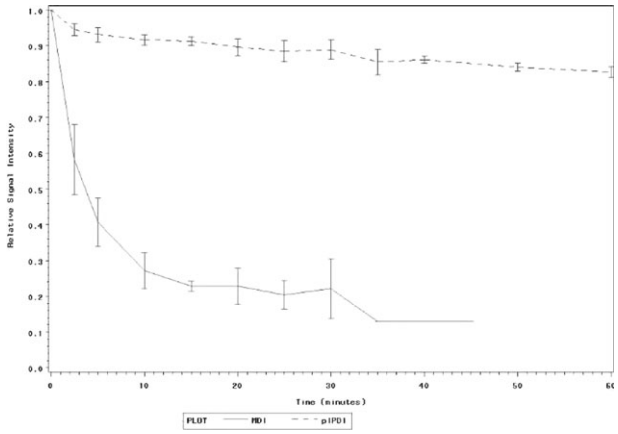


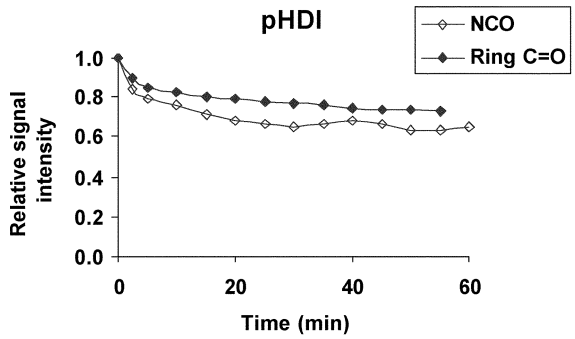
Fig. 5 Average change in the absorbance intensity of the N═C═O group at ~2270 cm^{-1} for MDI (0.21 μmol NCO cm^{-2}, $n = 4$) and pIPDI (0.27 μmol NCO cm^{-2}, $n = 5$). The line connects mean values of replicates; it is not a fitted line. Error bars represent ±1 standard deviation from the mean.

values of replicates at each time point. They are not regression-fitted lines. The concentration of free NCO groups on the skin surface declines following an exponential decay curve, very fast in the first few minutes then at a slower rate. On average, the NCO absorbance for octyl isocyanate decreased to 45% of the original amount in the first 5 min, to ~30% in 10 min and leveled off at ~15% (85% decrease) after 30 min (Fig. 4). For the aromatic, faster reacting MDI (Fig. 5), the average NCO absorption during the first 5 min decreased to ~40%, and at the end of 10 min it reached ~28%. After 30 min the MDI amount left was ~15% (85% decrease). The MDI peak height after this point approached the detection limit of the instrumentation and no further reliable measurements could be made. At higher MDI concentration (2.1 μmol NCO cm^{-2}, Fig. 6, case b) 43% of the original isocyanate group was still present on the surface after 1 h, suggesting a potential saturation effect.

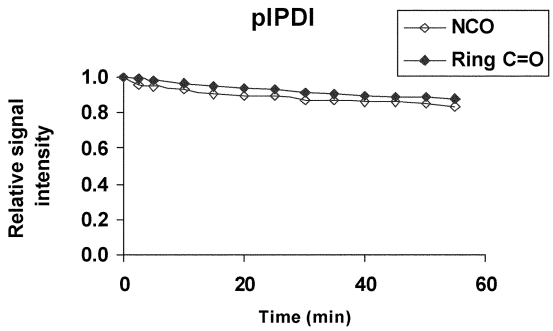
The behavior of polymeric isocyanates pHDI and pIPDI is in sharp contrast with octyl isocyanate and MDI. For pHDI (Fig. 4), the NCO absorbance decreased by 10% on average in the first 5 min, and remained nearly constant at 80% (20% decrease) after 30 min and changed little after 2 h (one observation, data not shown). A similar picture is evident from Fig. 5 for pIPDI. The NCO response decreased, on average, 7% in the first 5 min to 9% in 10 min, and changed very little after 30 min to reach ~15% decrease at the end of 60 min and 20% (80% remaining) 2 h later (one case, data not shown). The diffusion of pHDI is slightly faster than pIPDI, but the two are very comparable.

The variability in data is also considerable. There are at least two major factors which contribute to this variability; variation in the real concentration of isocyanates on the measured spot and the variability in the permeability of the stratum corneum. Although the average concentration of each isocyanate on the HL-GP skin was the same, the real concentration at the measured spot varied by as much as a factor of 3 (based on the NCO peak height at time zero). It is also known that the SC exhibits horizontal and vertical inhomogeneity in

A. pHDI for the case of 37% reduction in NCO over 1hr presented in Figure 3.



B. pIPDI; 17% reduction in NCO over 1 hr.



C. MDI; a = 0.2 μmol NCO/cm^2; b= 2.1 μmol NCO/cm^2.

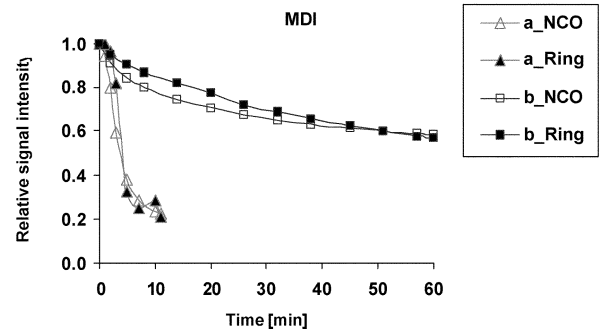


Fig. 6 Comparison of changes in spectral intensities for the absorbance bands at ~2270 (N=C=O) and ~1680 cm^{-1} (ring C=O of pHDI and pIPDI) or 1523 cm^{-1} (MDI ring) for pHDI (A), pIPDI (B), and MDI (C). The very comparable decay rates of the NCO and the ring C=O suggest that chemical reactions take place on a limited scale and these isocyanates leave the uppermost layers of the stratum corneum primary by diffusion.

the lipid content and hydration levels, which may play an important role in skin permeability.

Reaction *versus* permeation

The reduction in the NCO absorbance band indicates a decrease of the free NCO groups on the skin surface, which could be attributable to two different fates: chemical reaction with the various functional groups (OH, NH, SH) of the skin protein (mostly keratin) or other components of the lipid bilayers (ceramides, free fatty acids, *etc.*), or diffusion into deeper layers of the SC. It is presumed that if the NCO group chemically reacts with the skin, this reaction will covalently bind the molecule. In this case the absorbance of NCO group will decrease because NCO groups are being reacted, whereas an absorbance feature of the molecule (other than NCO) that is unreactive (*e.g.* the C=O stretch at ~1680 cm^{-1} for the isocyanurate ring) will remain constant, because the molecule is bound. Alternatively, if diffusion dominates then both the NCO absorbance and the isocyanurate ring C=O absorbance will be reduced simultaneously. Therefore, information can be gathered about the rate of these two processes (chemical reaction or diffusion) by comparing the rates of change of the ~2270 cm^{-1} and ~1680 cm^{-1} absorption bands over time. This approach can be used for pHDI and pIPDI, because they have a prominent absorbance due to the C=O group of the isocyanurate, and also for MDI, as the absorbance of the benzene ring (1523 cm^{-1}) can be used to monitor the fate of MDI. Octyl isocyanate does not have a prominent spectral feature that can be used for this purpose.

Fig. 6 compares the change in absorbance of NCO group *vs.* that of an unreactive part of the isocyanate molecule for pHDI (Fig. 6A), pIPDI (6B), and MDI (6C). For all three the NCO band and the band associated with the remainder of the molecule decrease at comparable rates, suggesting permeation into deeper layers of the skin. The NCO band disappears slightly faster than the other band, suggesting a minor chemical reaction of the NCO group, but this could explain only a small fraction of the total NCO loss.

Discussion

This FTIR study, which monitored the absorbance of the N=C=O group of 4 different isocyanates applied to hairless guinea pig skin, advances our understanding of the fate of isocyanate following skin exposure. Of note, all 4 isocyanates appear to penetrate the upper layers of HL-GP skin largely unreacted. However, there were substantial differences in the fate of the different isocyanates. The higher molecular weight (MW) polymeric isocyanates (pHDI and pIPDI) remained on the surface of the HL-GP skin much longer than small MW octyl isocyanate and MDI.

There is limited animal and human data for isocyanates to compare to these findings. Leibold *et al.*[21] applied a single dose of ^{14}C-labeled MDI on the skin of male Wistar rats and investigated the dermal uptake and systemic distribution of radioactivity. They found very low dermal uptake in plasma and other organs (<1%). Most of the applied dose was found at the application site, but less than 1% of radioactivity could be recovered from the skin with washes at the end of the 8 h period. The systemic uptake was increased to ~25%, when the isocyanate was administered intradermally. These data indirectly suggested that the vast majority of MDI was bound to the skin at the application site.[22] While radiolabelling of MDI enables monitoring the fate of the whole isocyanate molecule, it does not provide information on the fate of the NCO group. Wisnewski *et al.*[23] identified HDI bound to human keratin as the major HDI-bound protein following skin biopsies of human subjects epicutaneously exposed to 0.1% HDI in acetone, although the origin of the HDI-bound protein within the human skin could not be identified.

Human biomonitoring studies have documented urine and blood biomarkers of isocyanate exposure in work places where measured airborne exposures were very low and exposures were felt to be predominantly dermal, but have not documented dermal exposure or its fate.[24–26]

We are not aware of prior studies using FTIR to assess dermal isocyanate exposure and absorption, but FTIR has been used to assess other exposures, such as pesticides. Carden *et al.*,[27] found by a similar ATR-FTIR technique that ~10% of the original amount of pesticide (captan, and azinphos-methyl, average 3 and 5 μg cm^{-2}, respectively) was absorbed into the skin of human volunteers within 22 min, very similar to kinetics of pHDI and pIPDI on the HL-GP skin. They also observed a similar exponential curve for skin absorption of pesticides and comparable variability to our HL-GP skin data.

This study demonstrates the feasibility of ATR-FTIR to monitor isocyanate skin exposures. Current methods to detect isocyanates on skin or surfaces are limited. Quantitative skin wiping, which has been used for isocyanates in auto body shops,[11] may underestimate exposure due to skin porosity (lower recoveries) and skin absorption of isocyanates, especially for fast absorbing isocyanates. Qualitative skin wipes (SWYPES™, Colormetric Laboratories, Des Plains, IL) do not have sufficient sensitivity and their usefulness is restricted to the highest exposures. ATR-FTIR may be advantageous in evaluating skin decontamination and wipe sampling efficiency, for dermal exposure measurements and kinetic studies of isocyanate dermal absorption in humans. The ATR-FTIR measurements are considerably less expensive than the current high performance liquid chromatography (HPLC) measurements of skin wipes, which additionally require highly skilled analysts. Flexible portable ATR-FTIR instruments are commercially available and may be applicable to field studies. Additionally, the ATR-FTIR technique provides rapid real-time data, requires minimum sample preparation thus enabling collection of large amounts of data, is non-invasive and safe to the end user, and allows on-site measurements.

The study has several limitations: the first relates to the use of *in vitro* previously frozen HL-GP pig skin, and the second to the limited penetration depth (~1 μm) of the IR beam into the SC. The most relevant data eventually should be obtained *in vivo* with human volunteers, but ethical and medical issues related to isocyanate sensitization and the pilot nature of this work, restricted these initial studies to animal skin. Instrumental configuration did not allow direct measurements on living animals and previously frozen HL-GP skin was chosen partly because of availability. The primary concern with previously frozen HL-GP skin is whether these data can be used to make inferences about the behavior of isocyanates on living human skin. Factors which may limit the application of these findings to humans are that HL-GP skin SC is thinner than in humans, freezing the skin may have altered the permeability characteristics of the skin, and lack of blood circulation and metabolism also may influence the kinetics of skin absorption.

Because the HL-GP SC is considerably thinner than human SC, the IR beam penetrates deeper into the HL-GP SC. On the contrary, the IR beam would penetrate only the uppermost layer of the human SC (~1 μm), whose thickness (when dry) is 10–15 μm. This would restrict the FTIR investigation of human skin only to the outermost layers of the stratum corneum. Whether what is observed on the upper 1 μm holds for deeper layers of the human SC is less certain, albeit possible.

Another issue is the concentration of isocyanate used and the diluent. Concentrations used in this study are comparable to the upper percentile of measured skin and surface exposures in auto body shops (90%-ile for surface wipes >0.16 μg NCO cm^{-2}, our group's unpublished data). However, significantly higher skin concentrations can be reached in the field when the skin comes in contact with droplets of isocyanate material or highly contaminated surfaces. Pure isocyanate was diluted in ethyl acetate. Ethyl acetate is unlikely to effect the FTIR results, because the extremely small amounts used (30 μL) were allowed to evaporate (within about 15 s) prior to taking measurements. Additionally, evaporative losses of isocyanates as a potential source of error were studied separately by monitoring absorption of NCO groups of isocyanates spiked on the ATR crystal over one hour with and without an impermeable (aluminum foil) membrane (which mimicked the skin barrier) covering the ATR crystal. No evaporative losses were seen for pHDI and pIPDI and loses were of no practical importance for MDI. Thus loses due to evaporation of MDI from the uncovered ATR crystal at 24 °C, at comparable concentrations with those used for skin measurements, were 10% at 30 min and 20% at 60 min, and occlusion of the ATR crystal reduced evaporative losses to <5% in 60 min. Evaporative loses may have been greater for the volatile octyl isocyanate, and experiments suggest loses likely were in the range 5–15%. It is unlikely for such small losses to have biased the results significantly.

In the work place, isocyanates are rarely used alone and typically are mixed with polyols, solvents, catalysts, and other additives. Isocyanate reactivity with polyols during curing and the presence of solvents is expected to further complicate the kinetics of isocyanate absorption through the skin. We are planning to investigate such reactive systems in the field in conjunction with kinetic studies of isocyanate skin absorption on humans. Better qualitative and quantitative information on isocyanate skin absorption can be obtained from a combination of repeated tape striping and FTIR, which allows measuring the rate and depth of isocyanate penetration through the skin. Future laboratory and field testing of human skin exposure to isocyanates is necessary.

Conclusion

ATR-FTIR was used to investigate *in vitro* the fate of isocyanates on HL-GP skin. The low molecular weight octyl isocyanate and MDI disappeared from the outermost layer of the HL-GP skin relatively fast (~70% of the applied dose in 10 min), whereas for the higher molecular weight products, pHDI and pIPDI, the kinetics were much slower (~10% in 10 min). The FTIR data also indicated that these isocyanates penetrated the upper layers of the HL-GP SC largely unreacted. The ATR-FTIR appears to be a promising technique for *in vivo* studies of the kinetics of NCO absorption in humans and field measurements. More importantly, this

technique can be used to investigate the effect of various determinants (skin hydration, lipid content, solvent effect, penetration enhancers, barrier creams, *etc*.) on NCO penetration through the skin as well as decontamination efficiency for various skin decontamination products.

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