Quantification of Skin Acrylate Adducts
Grant No. K01OH00174-01

Closeout Report
18 December, 2001

Principal Investigator: Leena A. Nylander-French

School of Public Health
University of North Carolina
Chapel Hill, NC 27599-7400

ABSTRACT

This project aimed to develop a noninvasive procedure for collection of an epidermal tissue sample and to detect the deposition and penetration of a multifunctional acrylates into the stratum corneum and the formation of acrylate-keratin protein adducts as a biomarker of exposure.

Although a reliable and reproducible tape-stripping procedure was developed for tripropylene glycol diacrylate (TPGDA) exposed human skin, synthesis of a TPGDA-protein haptenic complex failed to produce epitope specific antisera for quantification. Two different methods to produce antisera failed, most likely, due to cross-linking of difunctional acrylate to multiple nonspecific sites. Therefore, an alternative procedure was adopted in order to accomplish the principal goals of this project. The alternative procedure focused on the preparation of a conjugated haptenic-peptide synthesized from benzene and naphthalene. Conjugated haptenic-peptides synthesized from benzene or naphthalene (adduction to form S-phenylcysteine) substituted cysteines complexed to keyhole limpet hemocyanin (KLH) were synthesized and the antibodies produced using New Zealand white rabbits. This successful demonstration of an antigenic epitope using this method provides a model for synthesis of an acrylated cysteine for synthesis of an antigenic keratin-1 or keratin-10 peptide for preparation of an immunogenic complex.

In addition, a reliable and reproducible tape-stripping procedure for sampling and isolation of naphthalene-keratin adducts (antigen) from the stratum corneum under defined laboratory conditions was developed and tested on human volunteers exposed to jet fuel instead of TPGDA. Keratin concentration as well as naphthalene concentration (as a marker for jet fuel exposure) from the adhesive tape strips were determined. This method can be used for isolation of keratin protein for identification and quantification of adducts by an enzyme-linked immunoabsorbent assay (ELISA).

We will continue the project by validating the developed methods under occupational exposure settings. This research can be extended to make it possible to define the dermal exposure to any hazardous chemical that reacts with keratin-rich epidermis, in either occupational or environmental exposure conditions. The results increase our knowledge of the correlation of dermal exposure with both the dermal dose and the potential dermal and systemic effects.

SIGNIFICANT FINDINGS AND USEFULLNESS OF FINDINGS

Development of a rapid and noninvasive sampling procedure using an adhesive tape stripping of the stratum corneum of the epidermis for sample removal and extraction and isolation of keratin suitable for identification and quantification of adducts by the ELISA method was completed successfully. Both multifunctional acrylates and keratin concentration for normalization of recovered multifunctional acrylate can be determined using analytical chemistry from the adhesive tape strips and future work will focus on the isolation of hapten-keratin adducts suitable for identification and quantification of adducts by an ELISA-method.

We were unable to synthesize a TPGDA-protein haptenic complex and, therefore, failed to produce epitope specific antisera with two different methods. An alternative procedure was adopted in order to accomplish the principal goals of this project. The alternative procedure focused on the preparation of a conjugated haptenic-peptide synthesized from benzene oxide and naphthalene (adduction to form S-phenylcysteine) substituted cysteines complexed to keyhole limpet hemocyanin (KLH). Successful demonstration of an antigenic epitope using this method

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

tripropylene glycol diacrylate, TPGDA; keyhole limpet hemocyanin, KLH; enzyme-linked immunoabsorbent assay, ELISA; keratin-1, KRT1; keratin-10, KRT10; immunoglobulin G, IgG; 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride, EDC; N,N-dimethylformamide, DMF; 9-fluorenylmethoxycarbonyl, FMOC; time-of-flight, TOF; matrix-assisted laser desorption ionization, MALDI; phenylmethyl sulfonyl fluoride, PMSF.

PUBLICATIONS

Pier Reviewed Publications

Copies of these papers are included in this Closeout Report.

1. Nylander-French, L.A: A Tape-Stripping Method for Measuring Dermal Exposure to Multifunctional Acrylates. Ann. Occup. Hyg. 44(8): 645-651, 2000.
2. Nylander-French, L.A.; Lacks, G.D.; Mattorano, D.A.: Quantification of naphthalene dermal exposure using non-invasive tape stripping of the stratum corneum, extraction, and normalization against keratin. Arbete och Hälsa 10:23-24, 2001.
3. Nam, T.-G.; Sangaiah, R.; Gold, A.; Lacks, G.D.; Nylander-French, L.A. and French, J.E.: Synthesis of FMOC-protected *S*-arylcysteines and a modified keratin sequence. Polycyclic Aromatic Compounds (in press).
4. Nylander-French, L.A.: Occupational hazards from the use of ultraviolet radiation curable acrylates. Occupational Safety and Health, Croner Publications (in press).

Abstracts and Presentations

1. Nylander-French, L.A.; Lacks, G.D.; and Mattorano, D.A. Quantification of naphthalene dermal exposure using non-invasive tape stripping of the stratum corneum, extraction, and normalization against keratin. X2001 – Exposure Assessment in Epidemiology and Practice, Göteborg, Sweden, June 10-13, 2001.
2. Nam, T.-G.; Sangaiah, R.; Gold, A.; Lacks, G.D.; French, J.E. and Nylander-French, L.A.: Synthesis of *S*-aryl-modified cysteines, FMOC derivatives and peptides of keratin 10 containing the modified cysteine epitope for polyclonal antisera production. Superfund Basic Research Program, Oxidative Processes: Stress to Remediation, Chapel Hill, North Carolina, December 12-14, 2000.
3. Nylander-French, L.A.: A dermal sampling method for measuring skin exposure to multifunctional acrylates. American Industrial Hygiene Conference and Exposition, Orlando, Florida, May 22-25, 2000.
4. Mattorano, D.A. and Nylander-French, L.A.: Predicting dermal exposure to jet fuel (naphthalene) using an adhesive tape-stripping method. American Industrial Hygiene Conference and Exposition. 20-25 May 2000, Orlando, Florida, USA.

Thesis Supervised

1. Mattorano, Dino A.: Estimating Dermal Exposure to Jet Fuel (Naphthalene) Using an Adhesive Tape Stripping Technique. Master's Thesis. August, 2000. Committee: Leena A. Nylander-French, Chair and Adviser; Stephen M. Rappaport, Ph.D., Professor, Environmental Sciences and Engineering; Lawrence L. Kupper, Ph.D., Professor, Biostatistics.

Papers to be submitted

Because of the difficulty in the synthesis of the TPGDA-keratin antibody and, therefore, the cancellation of human volunteer study with TPGDA, the completion of this research projects as originally submitted was not possible. However, the alternative procedure proposed facilitated accomplishment of the main goals of this project. Therefore, a number of papers are anticipated and will be submitted to appropriate journals in the next 3 – 4 months. We anticipate the following submissions:

1. Chao, Y and Nylander-French, L.A. Development of an Adhesive Tape-Stripping Technique to Measure Dermal Exposure to Naphthalene and Jet Fuel.

2. Nylander-French, L.A.; Lacks, G.D. and Chao, Y. Determination of Keratin Protein in a Tape-Striped Skin Sample from Jet Fuel Exposed Skin: Standardization of the Tape-Stripping Method.
3. Nylander-French, L.A. Dermal Exposure to Jet Fuel in Air Force Fuel-Cell Maintenance Workers.
4. Nylander-French, L.A.; Gold, A.; Nam, T.-G.; Sangaiah, R.; Lacks, G.D. and French, J.E.: Synthesis of *S*-Aryl-Modified Cysteines, FMOC Derivatives and Peptides of Keratin 1 and 10 Containing the Modified Cysteine Epitope for Polyclonal Antisera Production.

should provide a model for synthesis of an acrylated cysteine for synthesis of an antigenic keratin-1 (KRT1) or keratin-10 (KRT10) peptide for preparation of an immunogenic complex.

Target immunogenic peptides have been successfully synthesized incorporating *S*-phenylcysteine. The antibodies produced were affinity purified and the results for KRT1 yielded 0.9 mg of antibody and for KRT10 yielded 1.4 mg of antibody. Due to less than expected recovery of affinity purified IgG antibody, we will repeat both the peptide synthesis for antigen preparation and immunization to produce the required amount of high titer antibodies with strong avidity to the epitope prepared. We will continue the development of this promising technique using hapten-specific keratin adducts to determine dermal exposure.

SCIENTIFIC REPORT

Specific Aims

The following specific aims were proposed for this investigation:

Specific Aim #1. Develop and produce rabbit polyclonal antisera to TPGDA-keratin haptenic complex for identification of TPGDA-keratin adducts isolated from the keratinized layers (stratum corneum) of the skin for use in an ELISA method.

Specific Aim #2. Develop a rapid and noninvasive sampling procedure using an adhesive tape stripping of the stratum corneum of the epidermis for sample removal and extraction and isolation of TPGDA-keratin adducts suitable for identification and quantification of adducts by the ELISA method.

Specific Aim #3. Develop an ELISA method using the affinity purified IgG fraction of the polyclonal antisera for identification and quantification of the TPGDA-keratin adducts isolated from the stratum corneum of the TPGDA exposed skin.

Specific Aim #4. Test, evaluate, and validate the skin sampling procedure and the ELISA method for detection and quantification of TPGDA-keratin adducts by exposing the volar regions of the forearms of volunteers to precise nonsensitizing concentrations of TPGDA under controlled laboratory conditions.

Studies and Results

Specific Aim #1

Develop and produce a rabbit polyclonal antisera to TPGDA-keratin haptenic complex for identification of TPGDA-keratin adducts isolated from the keratinized layers (stratum corneum) of the skin for use in an ELISA method.

The first goal, as stated in the proposal, was the preparation of the TPGDA-keratin haptenic complex (immunogen) to induce polyclonal antisera in rabbits. As described in the previous Application for Continuation Grant, during the first year of this grant, the production of TPGDA-haptenic complexes was first pursued by adducting TPGDA with purified human keratin or albumin and purifying the resulted complex. Using this approach, we were unable to identify purified TPGDA-protein (either albumin or epidermal keratin) complex for use as an immunogen for PCA production. Albumin alone produced a band of approximately 66 kDa and albumin with TPGDA produced a series of bands greater than 66 kDa. There was no difference between keratin and keratin mixed with TPGDA. Because of the multiple bands produced by the albumin/TPGDA complex and the lack of unique bands for the keratin/TPGDA complex, another approach was necessary.

During year II, the alternative procedure focused on the preparation of a conjugated TPGDA-haptenic polypeptide alone or complexed to KLH. Under reducing conditions, TPGDA was adducted to purified synthetic polypeptides of selected sequences of KRT10 or human serum albumin. For KRT10, the exposed head region (residues 17 through 30; N-GGGGGGGGCGGGGG-COOH) was synthesized. Likewise, the selected amino acid sequence of human serum albumin, N-ALVLIAFAQYLQQCPFEDHV-COOH polypeptide (residues 45 through 64) was synthesized. Sequences for both polypeptides were selected because they presented a single cysteine residue with a free sulfhydryl as a nucleophilic target at the exposed surface of the parent macromolecule. Both polypeptides were conjugated to TPGDA under reducing conditions through nucleophilic substitution (Michael's addition). Each haptenic complex (TPGDA-KRT10 or TPGDA-albumin peptide) was conjugated to KLH (3 mg each) to enhance immunogenicity via the polypeptide carboxyl group using the EDC (1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride) method. Immunization was carried out at 8 subcutaneous sites using a priming inoculation of 0.5 mg protein in 0.1 ml complete Freund's adjuvant after obtaining 10 ml whole blood (pre-immune serum). At two-week intervals, subsequent immunization was carried out at same protein concentration in incomplete Freund's adjuvant. Fifty days post-priming, 50 ml of blood was removed from the ear vein for preparation of crude serum. Antibody titers of crude sera were not different between KRT10 or albumin peptide alone and TPGDA-KRT or TPGDA-albumin complex, respectively, indicating a lack of epitope specificity for either target peptide.

Protein structure analysis indicated that the selected KRT10 peptide of the head region fits a coiled structure of low hydrophilicity and neutral antigenicity. If adduction of the single cysteine residue on this sequence failed, the low solubility and antigenicity would be insufficient for an epitope specific immunogenic response. However, the albumin residue selected, which contains only a single cysteine residue as a target for adduction, should be a reasonable target. Protein sequence analysis predicts that the first 12 residues form a alpha-helix, while residues 13-20 form a random coil. The peptide varies from hydrophilic at the N-terminus to slightly hydrophobic at the C-terminus. Antigenicity is variable, but is reasonably high for this peptide. New target sequences are not an option due to the limited residues available on either of these two target proteins that provide nucleophilic targets.

Alternative Approach

Since preparation of TPGDA-protein haptenic complex failed to elicit epitope specific antisera with the two methods described above, we have adopted a final alternative procedure in order to accomplish the goals of this project. The alternative procedure focuses on the preparation of a conjugated TPGDA-haptenic peptides synthesized from TPGDA substituted cysteines complexed to KLH. Since we are working on Superfund Project (NIEHS, Project No. 2 P42 ES05948-09) to demonstrate skin adducts to benzene oxide and naphthalene (adduction to form S-phenylcysteine), we proposed to first test benzene oxide adduction to KRT10 or albumin peptide as a "proof of principle". Successful demonstration of an antigenic epitope using this method will provide a model for synthesis of an acrylated cysteine for synthesis of an antigenic KRT10 or albumin peptide for preparation of an immunogenic complex.

Initial work began with attempting to repeat the HPLC chromatogram provided with the KRT10 peptide of the head region commercially ordered from Sigma-Genosys (The Woodlands, TX). The synthetic peptide was found to be soluble in 0.1% acetonitrile in water. The column was YMC ODS-AQ, C-18, 3 m, 120 A, 4.0 X 50 mm and the mobile phase was A = 0.1% TFA/Water and B = 0.1% TFA/Acetonitrile. The gradient was 0 to 2 min 100% A and 2 to 20

min 0 to 60% B. The detection wavelength was 214nm. The chromatogram produced was similar to that provided by Sigma Genosys and it was found that the peptide, once put into solution, was unstable at room temperature.

Benzene oxide reaction with cysteine produces an S-phenylcysteine residue, which can be synthesized. We synthesized the KRT10 peptide substituting S-phenyl cysteine for complexing to KLH via the C-terminal reaction to produce an immunogen. The benzene oxide, because of its highly reactive state, was kept on dry ice or at -70°C. In a scintillation vial, the KRT10 was mixed with 1 ml of N,N-Dimethylformamide (DMF) and 100X molar concentration of benzene oxide. The vial was put into a boiling methanol bath and onto a rotary stirrer in a 4°C cold room for 1 hour. The solution was then stirred for an additional two hours at room temperature. The resulting product was lyophilized by speed vac for 2 hours.
The product was analyzed by electron spray ionization (ESI) rather than HPLC in order to quantitatively determine the product size. The molecular weight of the peptide is 862.839 and the addition of the benzene oxide adduct should add 76 to the mass, so a product of approximately 938 should be seen in the chromatograph. The ESI conditions were as follows: probe position at 1, aux. Gas at 80, sheath gas at 40, cap, temp. at 350°C and the mobile phase was acetonitrile/water/0.1% and AcH at 50 μl/min. Various injections were performed to see if any product was formed. However, the major product was 863.5 that corresponds to unreacted peptide.

Because of the inability of the KRT10 to go completely in solution using DMF, a synthetic peptide was made using S-phenyl cysteine. The assumption made was that the mixing of KRT10 and benzene oxide produces an S-phenyl cysteine adduct. The S-phenyl cysteine was made in our laboratory at UNC-Chapel Hill and then was shipped to Sigma Genosys for synthesis into KRT10. The prepared synthetic peptide was used as an antigen for the production of antibodies.

S-phenyl cysteine production
Since the lability of both benzene oxide and naphthalene-1,2-oxide in aqueous buffer precluded direct modification of KRT 10, the strategy adopted was to synthesize appropriately modified oligopeptide sequences using the FMOC-protected *S*-aryl cysteines. Since benzene oxide is symmetric, only the single adduct, *S*-phenylcysteine, is generated. Naphthalene-1,2-oxide may undergo nucleophilic attack at either position 1 or 2 to yield *S*-(1-naphthyl)cysteine and *S*-(2-naphthyl)cysteine respectively. Oligopeptides representing head regions of KRT1 and KRT10 were selected as appropriate immunogens. Oligopeptides containing a single modified cysteine were selected the synthetic targets: GGRFSS(C*)GG for KRT1 and GGGG(C*)GGGGG, for KRT10, where C* represents the appropriate *S*-aryl cysteine. Synthesis of the modified cysteines was accomplished by a high-yield Michael addition of the arylthiols to acetamidoacrylic acid to give the *S*-aryl mercapturic acids.

The mercapturic acids proved to be difficult to purify by crystallization or chromatography. However, it was possible to take advantage of the sparing solubility of the mercapturic acids in organic solvents to obtain very pure products by washing 5% bicarbonate solutions with ether, and then precipitating the mercapturic acid by carefully lowering the pH to 3with concentrated hydrochloric acid. Deacetylation of the mercapturic acids proved to be challenging, because, like the *S*-arylmercapturic acids, the *S*- arylcysteines could not be recrystallized or conveniently purified in quantity by chromatographic techniques. Methanolic ammonia was not effective and refluxing with HCl in the presence of unhindered alcohols was precluded by the likelihood of esterification of the carboxylic acid function. However, this

problem was circumvented by extended refluxing in 1:1 *t*-butanol/concentrated HCl under an inert atmosphere, with progress monitored by ^{1}H NMR spectrometry. The *S*-arylcysteines could be isolated by evaporation of the reaction solution. The 9-fluorenylmethoxycarbonyl (FMOC) derivatives were synthesized by a published procedure, modified because of the low solubility of the *S*-aryl adducts of cysteine. The FMOC derivatization was done on the adducts as a slurry and the reaction time was doubled. By products arising from side reactions of the 9-fluorenylchloroformate reagents were removed from the aqueous reaction mixture by ether extraction. The pure FMOC derivatives could then be isolated as crystalline solids by careful adjustment of the pH with hydrochloric acid.

To date, target immunogenic peptides have been synthesized incorporating *S*-phenylcysteine. The oligopeptides were synthesized commercially by Sigma-Genosys, and have been characterized by HPLC retention times and time-of-flight (TOF) mass spectra acquired using matrix-assisted laser desorption ionization (MALDI) source.

Antibody production

The synthetic peptides were used to make KRT1 and KRT10 KLH-synthetic peptide conjugates. Polyclonal antisera was produced by multiple subcutaneous injections of the mixed conjugates in complete Freund's adjuvant (CFA) to the dorsal skin of two New Zealand white rabbits. Pre-immune serum was collected prior to the start of injections and immunizations were performed every seven days with production bleeds occurring every 2 weeks. The experiment ran for 18 weeks and all production bleeds were allocated and frozen at –20°C. The final bleeds, acquired on August 1, 2001 were used to affinity purify the antibodies produced. The results indicated a yield of 0.9 mg and 1.4 mg of antibody for KRT1 and KRT10 by ELISA, respectively. Due to less than expected recovery of affinity purified IgG antibody, we will repeat both the peptide synthesis for antigen preparation and immunization to produce the required amount of high titer antibodies with strong avidity to the epitope prepared.

Specific Aim #2

Develop a rapid and noninvasive sampling procedure using an adhesive tape stripping of the stratum corneum of the epidermis for sample removal and extraction and isolation of TPGDA-keratin adducts suitable for identification and quantification of adducts by the ELISA method.

CoverRoll-tape (Beiersdorf, Germany), cut to 2.5 cm × 4.0 cm size, was used to collect keratin samples from the skin. After sample collection, the tape was rolled (sticky side to the outside) and placed into a 2 ml cryovial. First, the sample removal and isolation of keratin proteins from the tape strip was attempted by flash freezing the tape stripped skin sample in liquid nitrogen. The sample was removed from liquid nitrogen, kept on dry ice, and the cells scraped from the tape and put into a 15 ml polypropylene tube. The cells were homogenized in a solution of 25 mM Tris-HCl (pH 7.4) containing 1mM EDTA, 1 mM EGTA, 1 mM phenylmethyl sulfonyl fluoride (PMSF), antipain (10 μg/ml), pepstatin (5 μg/ml). The tubes were spun to remove air bubbles and the homogenate was placed into a 2 ml microcentrifuge tube and centrifuged (10,000 g for 10 min at 4°C). The supernatant was discarded and the pellet was extracted as follows: 1) Samples to be analyzed by SDS PAGE were extracted (95°C, 5 min) with 1% SDS, 25 mM Tris-HCL (pH 7.4) to obtain keratin, 2) after centrifugation, the pellets were further extracted with 1% SDS and 5% 2-mercapthoethalnol in 25 mM Tris-HCL (pH 7.4) to obtain keratins of the stratum corneum, and 3) samples to be analyzed on two-dimensional gels were similarly extracted, except that a fresh solution of 8 M urea replaced SDS and the extraction was carried out at room temperature.

After significant interference from reagents was observed, another method based on the Bradford assay was developed. After placing the rolled tape (with adhesive side out) into a 2 ml cryovial, one ml of 1M NaOH was added and the tube was vortexed at various intervals over for 2 h period. The extract was stored at 4°C overnight when required. Next, 1 ml 1M HCl was added to neutralize and the sample mixed by vortexing. The Bradford assay was performed according to Amresco™ protocol. A standard curve was prepared from human keratin obtained from Sigma™. Known concentrations of keratin standard obtained from Sigma™ were allocated into separate microcentrifuge tubes and a quantity of 1M NaCl was added to bring the volume to 100 μl total. One milliliter of Bradford reagent was added and the sample was vortexed and allowed to stand at room temperature for 2 minutes. The absorbance was determined by spectrophotometry at 595 nm using a 1ml microcuvette. Absorbance was plotted against protein concentration to generate a standard curve. Unknown samples were analyzed using the same procedure and the concentration determined using the prepared standard curve.

This successful completion of this aim has led to the development of a rapid and noninvasive sampling procedure using an adhesive tape stripping of the stratum corneum of the epidermis. Keratin concentration can be determined and future work will focus on the isolation of TPGDA-keratin adducts suitable for identification and quantification of the adducts by an ELISA method.

Specific Aim #3

Develop an ELISA method using the affinity purified IgG fraction of the polyclonal antisera for identification and quantification of the TPGDA-keratin adducts isolated from the stratum corneum of the TPGDA exposed skin.

Since the preparation of TPGDA-protein haptenic complex has failed to elicit epitope specific antisera with the two methods described above, we have not been able to work on Specific Aim #3. However, the ELISA method will be developed, as described in the study proposal, when epitope specific antisera have been produced for naphthalene and benzene.

Specific Aim #4

Test, evaluate, and validate the skin sampling procedure and the ELISA method for detection and quantification of TPGDA-keratin adducts by exposing the volar regions of the forearms of volunteers to precise nonsensitizing concentrations of TPGDA under controlled laboratory conditions.

Because the method development was not completed, no human volunteer exposures were performed. However, the progress made with the work performed in relation to this study has led to the naphthalene exposure analysis of jet fuel workers, via the tape stripping method coupled to a GC/MS analysis. Laboratory experiments using jet fuel on human volunteers have been completed and the data analysis is being competed and a manuscript is currently being prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service
Centers for Disease Control
and Prevention (CDC)

Memorandum

Date: March 28, 2002

From: Lee M. Sanderson, Ph.D., Program Official
Office of Extramural Programs, NIOSH, E-74

Subject: Final Report Submitted for Entry into NTIS for Grant 5 K01 OH000174-03.

To: William D. Bennett
Data Systems Team, Information Resources Branch, EID, NIOSH, P03/C18

The attached final report has been received from the principal investigator on the subject NIOSH grant. If this document is forwarded to the National Technical Information Service, please let us know when a document number is known so that we can inform anyone who inquires about this final report.

Any publications that are included with this report are highlighted on the list below.

Attachment
cc: Sherri Diana, EID, P03/C13

List of Publications

Nam T-G, Sangaiah R, Gold A, Lacks GD, Nylander-French LA, French JE: Synthesis pf FMOC-Protected S-Arylcysteines and A Modified Keratin Sequence. Polycyclic Aromatic Compounds, in press, 2002

Nylander-French LA, Lacks GD, Mattorano DA: Quantification of Naphthalene Dermal Exposure Using Non-Invasive Tape Strippig of the Stratum Corneum, Extraction, and Normalization Against Keratin. Arbete och Halsa 10:23-24, 2001

Nylander-French LA: A Tape-Stripping Method for Measuring Dermal Exposure to Multifunctional Acrylates. Ann Occup Hyg 44(8):645-651, 2000

Nylander-French LA: Occupational Hazards from the Use of Ultraviolet Radiation Curable Acrylates. Occupational Safety and Health, Croner Publications (in press), 2002

Title: Quantification of Skin Acrylate Adducts
Investigator: Leena A. Nylander-French, Ph.D.
Affiliation: University of North Carolina
City & State: Chapel Hill, NC
Telephone: (919) 966-3826
Award Number: 5 K01 OH000174-03
Start & End Date: 9/30/1998–9/29/2001
Total Project Cost: $162,000
Program Area: Allergic and Irritant Dermatitis
Key Words: biomarkers, skin, dermatitis

Abstract:
This project aimed to develop a noninvasive procedure for collection of an epidermal tissue sample and to detect the deposition and penetration of a multi functional acrylates into the stratum corneum and the formation of acrylate-keratin protein adducts as a biomarker of exposure.

Although a reliable and reproducible tape-stripping procedure was developed for tripropylene glycol diacrylate (TPGDA) exposed human skin, synthesis of a TPGDA-0protein haptenic complex failed to produce epitope specific antisera for quantification. Two different methods to produce antisera failed, most likely, due to cross-linking of difunctional acrylate to multiple nonspecific sites. Therefore, an alternative procedure was adopted in order to accomplish the principal goals of this project. The alternative procedure focused on the preparation of a conjugated haptenic-peptide synthesized from benzene and naphthalene. Conjugated haptenic-peptides synthesized from benzene or naphthalene (adduction to form S-phenylcysteine) substituted cysteines complexed to keyhole limpet hemocyanin (KLH) were synthesized and the antibodies produced using New Zealand white rabbits. This successful demonstration of an antigenic epitope using this method provides a model for synthesis of an acrylated cysteine for synthesis of an antigenic keratin-1 or keratin-10 peptide for preparation of an immunogenic complex.

In addition, a reliable and reproducible tape-stripping procedure for sampling and isolation of naphthalene-keratin adducts (antigen) from the stratum corneum under defined laboratory conditions was developed and tested on human volunteers exposed to jet fuel instead of TPGDA. Keratin concentration as well as naphthalene concentration (as a marker for jet fuel exposure) from the adhesive tape strips were determined. This method can be used for isolation of keratin protein for identification and quantification of adducts by an enzyme-linked immunoabsorbent assay (ELISA).

We will continue the project by validating the developed methods under occupational exposure settings. This research can be extended to make it possible to define the dermal exposure to any hazardous chemical that reacts with keratin-rich epidermis, in either occupational or environmental exposure conditions. The results increase our knowledge of the correlation of dermal exposure with both the dermal dose and the potential dermal and systemic effects.

Publications

Nylander-French LA: Occupational Hazards from the Use of Ultraviolet Radiation Curable Acrylates. Occupational Safety and Health, Croner Publications (in press), 2002

Nam T-G, Sangaiah R, Gold A, Lacks GD, Nylander-French LA, French JE: Synthesis pf FMOC-Protected S-Arylcysteines and A Modified Keratin Sequence. Polycyclic Aromatic Compounds, in press, 2002

Nylander-French LA, Lacks GD, Mattorano DA: Quantification of Naphthalene Dermal Exposure Using Non-Invasive Tape Strippig of the Stratum Corneum, Extraction, and Normalization Against Keratin. Arbete och Halsa 10:23-24, 2001

Nylander-French LA: A Tape-Stripping Method for Measuring Dermal Exposure to Multifunctional Acrylates. Ann Occup Hyg 44(8):645-651, 2000