FINAL PROGRESS REPORT

Title of Project:

Efficacy Study of a Nicotine Barrier Cream

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ABSTRACT

In September, 2013, the University of North Texas Health Science Center received the R03OH009815 grant from the CDC/NIOS and initiated the efficacy study of a nicotine barrier cream, with the goal of developing a barrier cream and assessing the efficacy, acceptability, side effects and ease of use in *in vitro* testing and in a pilot field study.

Aims: The main objective of this study was to develop a barrier cream and evaluate its efficacy in the laboratory and conduct a small field study to evaluate its efficacy in reducing nicotine exposure from tobacco harvesting workers in tobacco farms in Kentucky. Our specific aims were as follows: Aim 1: To develop a nicotine barrier cream and identify optimal formulations in the laboratory. Aim 2: To assess its efficacy in reducing dermal absorption of nicotine using *in vitro* testing. Aim 3: To evaluate its efficacy, acceptance, side effects and feasibility in a field pilot study.

Methods: The study included three closely related, but separate, parts designed to achieve the three specific aims: development and optimization of barrier cream formulations performed by Colormetric Laboratories, Inc.; efficacy study by *in vitro* testing conducted by the University of Maryland at Baltimore and pilot field study in tobacco farms with tobacco farm workers conducted by the University of North Texas Health Science Center (UNTHSC) in collaboration with researchers at the University of Kentucky at Lexington and the University of Texas at Dallas. The development of formulations was based on acidic components in the cream which were adjusted based on the *in vitro* testing to achieve optimal efficacy in reducing permeation of nicotine. The *in vitro* testing used miniature pig skin, a PermeGear flow-through diffusion cell system with L-nicotine solution and tobacco extract as nicotine donors and high performance liquid chromatography (HPLC) for analysis on permeated nicotine through the formulation-treated skin or control skin. Formulation-treated cotton gloves were also tested on their efficacy as a potential tool for skin protection. The field pilot testing recruited migrant tobacco farm workers, and used two best formulations from the *in vitro* testing to compare the efficacy between non-use and use time periods of the study week and between two formulations. Post-shift urine samples were collected from all workers and analyzed for urinary nicotine and cotinine adjusted for creatinine amount and body surface area. Two-sample t test, analysis of variance (ANOVA) and mixed effect modeling were used in the data analysis.

Results: Four formulations of the cream with various acid components were developed which were all found to reduce nicotine permeation *in vitro*. The best barrier cream formulations reduced *in vitro* skin permeation of nicotine by 97.6% from L-nicotine, by 64.0% from green tobacco leaf extract and by 86.6% from green tobacco leaf extract for gardening gloves coated with the barrier cream. A total of 43 workers from 6 farms participated in the field study. Gender, age, education, tasks performed during harvesting and barrier cream use, were identified as predictors of nicotine exposure. The nicotine formulations were well accepted by workers although the efficacy was not yet optimal.

Conclusion: The developed barrier cream formulations have the potential to reduce nicotine permeation through the skin. However, the current study is limited in its design and did not show its optimal efficacy. Further studies with improved design, large sample size and pre-shift urine samples to analyze for cross-shift comparison are recommended.

HIGHLIGHTS/SIGNIFICANT FINDINGS

Four formulations of the barrier cream showed significant reduction of nicotine through in *in vitro* testing.

Cotton gloves coated with one of the formulations (B+) showed high reduction of nicotine in *in vitro* testing

Gender, age, education, tasks performed during harvesting and barrier cream use, were identified as predictors of nicotine exposure in the pilot field study.

TRANSLATION OF FINDINGS

This study demonstrated the feasibility of translating our laboratory study results into the field practices. Although the efficacy of the barrier cream is not yet optimal the cream has the potential to be a useful, easy, feasible and acceptable tool of prevention to reduce nicotine exposure and the prevalence of GTS. It also demonstrates that we have the access to the tobacco harvesting worker population. We plan to continue to conduct a Phase II clinical trial study with a larger sample size to further evaluate the efficacy of the barrier cream in the field. We are also preparing a proposal to CDC/NIOSH to conduct a comprehensive intervention study so we could identify other effective prevention methods to reduce nicotine exposure and the prevalence of GTS.

OUTPUT/OUTCOMES /IMPACT

We have encouraged 6 participating tobacco farms and their workers to use more safe work practices, personal protective equipment and effective skin cleaners for reducing nicotine exposure. Farm owners in the study showed great interest and willingness to participate in future studies and workers learned the knowledge of skin protection in general and nicotine reduction in harvest work in particular.

We have through this study trained two Research Assistants who were Master of Public Health students at the UNTHSC and a Postdoctoral Research Associate at the University of Maryland. We have presented three posters at international conferences and submitted one manuscript. We have also received four awards for this study.

SCIENTIFIC REPORT

Background

Tobacco is a very important part of the agricultural economy and culture in Kentucky where it is grown in 104 of Kentucky's 120 counties. There are currently about 4,500 tobacco farms and approximately 60,000 persons harvesting tobacco annually at least part-time in the State. It is also grown in other parts of the U.S. and around the world. Tobacco farmers and farm workers can have significant skin exposure to and absorption of dissolved nicotine from wet tobacco leaves or saps during the harvesting work of tobacco plants, which can cause green tobacco sickness (GTS) or nicotine poisoning, a series of symptoms that may be so severe as to require emergency medical treatment. There have been published case reports of GTS in Kentucky, Florida, Tennessee and North Carolina as well as other parts of the world. Estimated prevalence rate varies from 1% to 66.3%. Despite the seriousness of the illness and large number of workers affected, very little research has been conducted to address the prevention of this disease and evaluate the effectiveness of strategies to reduce exposures to nicotine in tobacco harvesting workers, particularly in migrant and seasonal farm workers. Current preventive measures using protective clothing (e.g., chemical-resistant gloves or waterproof rain gear) and work schedule change may not be feasible and practical due to various limitations such as hot work environment and heat stress, busy work schedule and fast absorption of nicotine into the skin and blood. Alternative protective strategies and nicotine exposure prevention methods are therefore well needed and should be actively sought and field validated. Therefore, we proposed this study.

Specific Aims

The main objective of this study was to develop a barrier cream and evaluate its efficacy in the laboratory and conduct a small field study to evaluate its efficacy in reducing nicotine exposure from tobacco harvesting workers in tobacco farms in Kentucky.

Our specific aims were as follows:
Aim 1: To develop a nicotine barrier cream and identify optimal formulations in the laboratory.
Aim 2: To assess its efficacy in reducing dermal absorption of nicotine using *in vitro* testing.
Aim 3: To evaluate its efficacy, acceptance, side effects and feasibility in a field pilot study.

Methods Summary

This study included three closely related, but separate, parts designed to achieve the three specific aims. The first part – development and optimization of barrier cream formulations, was performed by Consultant, Mr. Thomas Klingner, in CLI Labs at Des Plains, IL. The second part – efficacy study by *in vitro* testing, was conducted by Dr. Audra Stinchcomb at the University of Maryland at Baltimore, MD. The third part – pilot field study in tobacco farms with tobacco farm workers, was conducted by the PI and his colleagues at the University of North Texas Health Science Center (UNTHSC) in collaboration with researchers at the University of Kentucky at Lexington and the University of Texas at Dallas. More details on materials and methods are provided in the Results for Specific Aims section below.

Results Summary

Four formulations of the cream with various acid components were developed which were all found to reduce nicotine permeation in vitro. The best barrier cream formulations reduced in vitro skin permeation of nicotine by 97.6% from L-nicotine, by 64.0% from green tobacco leaf extract and by 86.6% from green tobacco leaf extract for gardening gloves coated with the barrier cream. A total of 43 workers from 6 farms participated in the field study. Gender, age, education, tasks performed during harvesting and barrier cream use, were identified as predictors of nicotine exposure. The nicotine formulations were well accepted by workers although the efficacy was not yet optimal.

Methods and Results for Specific Aims

Aim 1: To develop a nicotine barrier cream and identify optimal formulations in the laboratory.

Methods:

Nicotine in tobacco exists as a lipophilic free base and readily penetrates the skin. Nicotine forms a water-soluble salt with almost any organic acid such as ascorbic or tartaric acid. Organic acids in the barrier cream would react with nicotine to form water soluble salts to reduce skin absorption of nicotine. Before the study, two formulations of the barrier cream were already developed. During the study, Co-I Thomas Klingner developed additional two formulations and a placebo formulation for *in vitro* testing. He worked with Co-I Dr. Audra Stinchcomb and her team members at the University of Maryland Baltimore during the *in vitro* testing to repeatedly modify the formulation components so optimal efficacy be obtained. He also worked with Dr. Stinchcomb's group to develop barrier cream-coated cotton gloves for the *in vitro* testing which would be potentially useful as additional protection tool for nicotine skin exposure intervention.

Results:

For preparation of Formulation A, 1.5 % w/v of methylcellulose was added to purified hot water (80-90°C) followed by the addition of citric acid (0.5% w/v) and tartaric acid (0.5% w/v). A pH of about 1 to 2 was observed. Potassium acetate (0.25% w/v) was added to increase the pH to 3. Polysorbate 80 was added as a surfactant/emulsifier. Formulation A+ was a more acidic formulation compared to Formulation A, with 1.5 % w/v of citric acid and 1.5 % w/v of tartaric acid. Methylcellulose amount was unchanged at 1.5% w/v and potassium acetate was increased to 0.7% w/v.

For preparation of Formulation B, 3.0% w/v of methylcellulose was dissolved in purified hot water (80-90°C). To this, citric acid (0.7% w/v), ascorbic acid (0.9% w/v) and tartaric acid (0.6% w/v) were added. The solution remained clear and had a pH of approximately 3. Polysorbate 80 was added as a surfactant/emulsifier. Formulation B+ contained ascorbic acid and citric acid each increased to 3.0% w/v, 1.6% w/v of methylcellulose and 1.0% w/v of potassium acetate.

Placebo formulation was also prepared for the *in vitro* testing in Specific Aim 2 to better demonstrate the effectiveness of the barrier creams. This formulation was prepared with 0.8% w/v of methylcellulose and excluded the ascorbic or citric acid, with a pH of 7.0.

Please see the *in vitro* testing section on the type of cotton gloves selected and coating methods.

Aim 2: To assess its efficacy in reducing dermal absorption of nicotine using *in vitro* testing.

Methods:

1) *In vitro* skin diffusion studies:

The Yucatan minipig skin was dermatomed to a thickness of approximately 250 µm using a Padgett® dermatome and then stored at -20°C until used. Stored skin samples were thawed to room temperature at the time of the experiment. Prior to placing the skin section in the diffusion cell, 40 µL of the investigational barrier formulation was gently rubbed onto the surface. In more detail, using a clean Teflon stir bar, the barrier cream was gently rubbed into the skin (0.95 cm^2) using a circular motion for approximately 30 seconds and then allowed to dry for approximately 20–30 min. This amount and time allowed adequate formulation coverage of the exposed skin area without leaving an undesirable pooling of the investigational formulation in the well of the diffusion cell, nor excess on the skin which would be undesirable for users of the formulation in the translational environment.

A PermeGear flow-through (In-Line, Hellertown, PA) diffusion cell system with diffusional area of 0.95 cm^2 was used for all *in vitro* skin studies. Skin surface was maintained at 32°C with a circulating water bath. Receiver solution was saline (0.9% w/v of high pressure liquid chromatography [HPLC] grade sodium chloride [NaCl] in water). Nicotine donor solutions consisted of L-nicotine in water at a concentration of 0.7 mg/mL or green leaf

tobacco extract. Five hundred µL of donor solution (either 0.7 mg/mL of L-nicotine or green leaf tobacco extract, containing 0.1 ± 0.05 mg/mL of nicotine) was placed on top of each pretreated skin section or the pretreated glove section. For control diffusion cells, just nicotine solution or green leaf tobacco extract solution was placed on the skin, without the barrier cream pretreatment.

2) Preparation of gloves coated with barrier cream:

Two types of gardening gloves were tested (Figure 1), thick knitted cotton gloves (brown gloves) and thin cotton protective gloves (white gloves). Blended cotton gardening gloves were selected since cotton-polyester blended clothing is what farmers' conventionally wear during harvest in warm weather. Both these gloves were immersed and soaked in Formulation B+ for 5 min and air dried prior to diffusion studies.

Brown gloves: String knitted cotton work gloves, medium size, medium weight, light brown color, 7 gauge, (purchased from amazon, Westchester 706S, elastic wrist cuff manufacturer: WestChester) (Figure 1A). The knit construction allows breathability with less hand fatigue. These gloves may be used for gardening, warehousing, parts handling, assembly work and food processing.

White gloves: Cotton inspector reversible/unhemmed gloves, medium weight, white color, 7 gauge (purchased from Amazon, MCR Safety 8600C, manufacturer: MCR safety (Figure 1B). These gloves can be used as protective liners for hands and ideal for light gardening and chores.

Both the brown and white gloves were immersed into a solution of Formulation B+ and soaked for 5 min before air-drying them. After being air-dried, they were stored in re-sealable bags until further use with diffusion experiments. Tests for protective efficiency of each glove type against tobacco extract was performed as explained in the previous section.

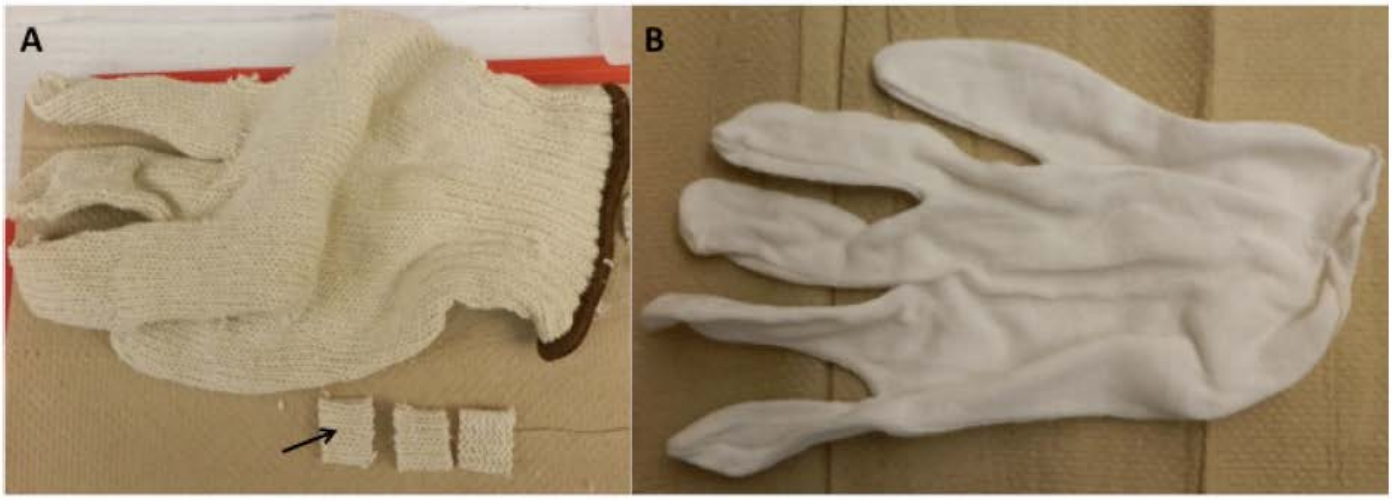

Figure 1. (A) String knitted 100% cotton work glove, medium size, medium weight, light brown color, 7 gauge (brown glove). Arrow shows the square section that is cut precisely to fit inside the flow-through diffusion cell and sits on top of the skin during diffusion. (B) Cotton inspector reversible/unhemmed glove, medium weight, white color, 7 gauge (white glove).

3) Sample analysis:

All nicotine samples were analyzed by HPLC consisting of a Waters (Milford, MA) Alliance Separations Module e2695 with column heater, a Waters 2489 dual absorbance detector set at a wavelength of 260 nm and Waters Empower™ software. Additionally, a Brownlee (Perkin Elmer®; Wellesley, MA) C-18 reversed-phase Spheri-5 µm column (220 x 4.6 mm) with a C-18 reversed phase 7 µm guard column (15 x 3.2 mm) was used. The mobile phase consisted of 30 mM ammonium acetate (with 2% acetonitrile):methanol (30:70) and flow rate of 1.0 mL/min. The injection volume was 100 µL, with a run time of 11 min and the retention time of nicotine was 5.8 min.

Diffusion samples were collected and analyzed by HPLC. Nicotine standards were prepared in 0.9% saline solution (0.9% w/v of HPLC grade NaCl in MilliQ filtered water). The limit of detection (LOD) (0.08 µg/mL) and limit of quantification (LOQ) (0.15 µg/mL) were determined. All samples and standards were injected in

duplicate. Working standard solutions were prepared fresh daily in the range of 0.08–10 µg/mL. All samples analyzed were within the standard curve range.

4) Data analysis:

The permeation data were plotted as cumulative amount of nicotine collected in the receiver compartment as a function of time. Lag time was determined by calculating the average of the x-intercepts upon extrapolation of the slope line from the cumulative values. The percent reduction of cumulative amount of nicotine was determined by using equation 1, where the percent difference between cumulative amounts at 24 h for treated skin and untreated skin was calculated for each formulation.

$$\text{Percent Reduction Cumulative Amount} = \frac{Cm_{skin} - Cm_f}{Cm_{Skin}} \times 100 \qquad (1)$$

Where Cm_{skin} is the cumulative amount of nicotine through skin at 24 h without treatment and Cm_f is the cumulative amount of nicotine at 24 h of skin treated with formulation (either by Formulation A, B, A+, B+). Statistical significance for nicotine diffusion through the skin was determined by one-way analysis of variance (ANOVA) and Tukey post-hoc analysis where $p<0.05$ was selected, using SigmaStat 3.5 software (San Jose, CA), to determine the significant differences between the tested barrier cream formulations.

Transepidermal water-loss (TEWL) measurements of the skin allowed for testing the integrity of the dermatomed skin (cyberDERM RG_1 Evaporimeter; Broomall, PA). TEWL values of <10 g/m^2h were regarded as acceptable values for the diffusion experiments.

Results:

1) L-nicotine permeation:

In this study we evaluated barrier cream Formulations A, B, A+ and B+. All barrier cream formulations showed a significant decrease ($p<0.05$) in nicotine permeation compared to untreated skin (Table 1 and Figure 2,). Formulations A, B, A+ and B+ showed a significant reduction in the cumulative amount of nicotine at 24 h (43.88 ± 34.17, 65.13 ± 42.33, 20.81 ± 24.47 and 3.74 ± 2.10 nmol, respectively) compared to untreated skin (153.41 ± 91.22 nmol) (Table 1). Formulations A+ and B+ were the most effective barriers for the L-nicotine (0.7 mg/mL) donor solutions and were selected for additional testing with green tobacco leaf extract. Lag time for cumulative permeation of nicotine through untreated skin was 0.9 ± 1.1 h; lag times for Formulations A, B, A+ and B+ were 1.8 ± 1.8 h, 1.3 ± 1.9 h, 7.0 ± 3.6 h and 7.7 ± 3.7 h, respectively (Table 1). Formulations A+ and B+ lag times were significantly longer ($p<0.05$), compared to the lag times of Formulation A, Formulation B and untreated skin. Formulations A and B lag times were not significantly different ($p>0.05$) compared to the untreated skin. The percent reductions in cumulative amounts at 24 h for Formulations A+ and B+ were considerably higher (86.4% and 97.6%, respectively) compared to the Formulations A and B (71.4% and 57.5%, respectively) for the 0.7 mg/mL L-nicotine donor solution.

Table 1 Barrier cream formulations tested against L-nicotine and green tobacco leaf extract

0.7 mg/mL nicotine as donor solution			
Applied formulations	**Cumulative amount at 24 h (nmol) Mean ± SD**	**Lag time (h) Mean ± SD**	**Percent reduction of cumulative amount at 24 h**
Formulation A (n=7)	*43.88 ± 34.17	1.8 ± 1.82	71.4 %
Formulation B (n=8)	*65.13 ± 42.33	1.3 ± 1.87	57.5 %
Formulation A+ (n=4)	*20.81 ± 24.47	*7.0 ± 3.56	86.4 %
Formulation B+ (n=3)	*3.74 ± 2.10	*7.7 ± 3.74	97.6 %
Untreated skin (n=7)	153.41 ± 91.22	0.9 ± 1.10	--
Nicotine from green tobacco leaf extract as donor solution			
Formulation A+ (n=9)	*29.66 ± 15.15	5.47 ± 1.13	59.5 %
Formulation B+ (n=8)	*26.40 ± 8.27	4.33 ± 2.02	64.0 %
Placebo (n=4)	95.64 ± 55.87	6.06 ± 1.67	0% (-30.6%)
Untreated skin (n=5)	73.24 ± 20.25	5.02 ± 0.78	--
*Designates significant difference (p<0.05) compared to respective untreated skin values			

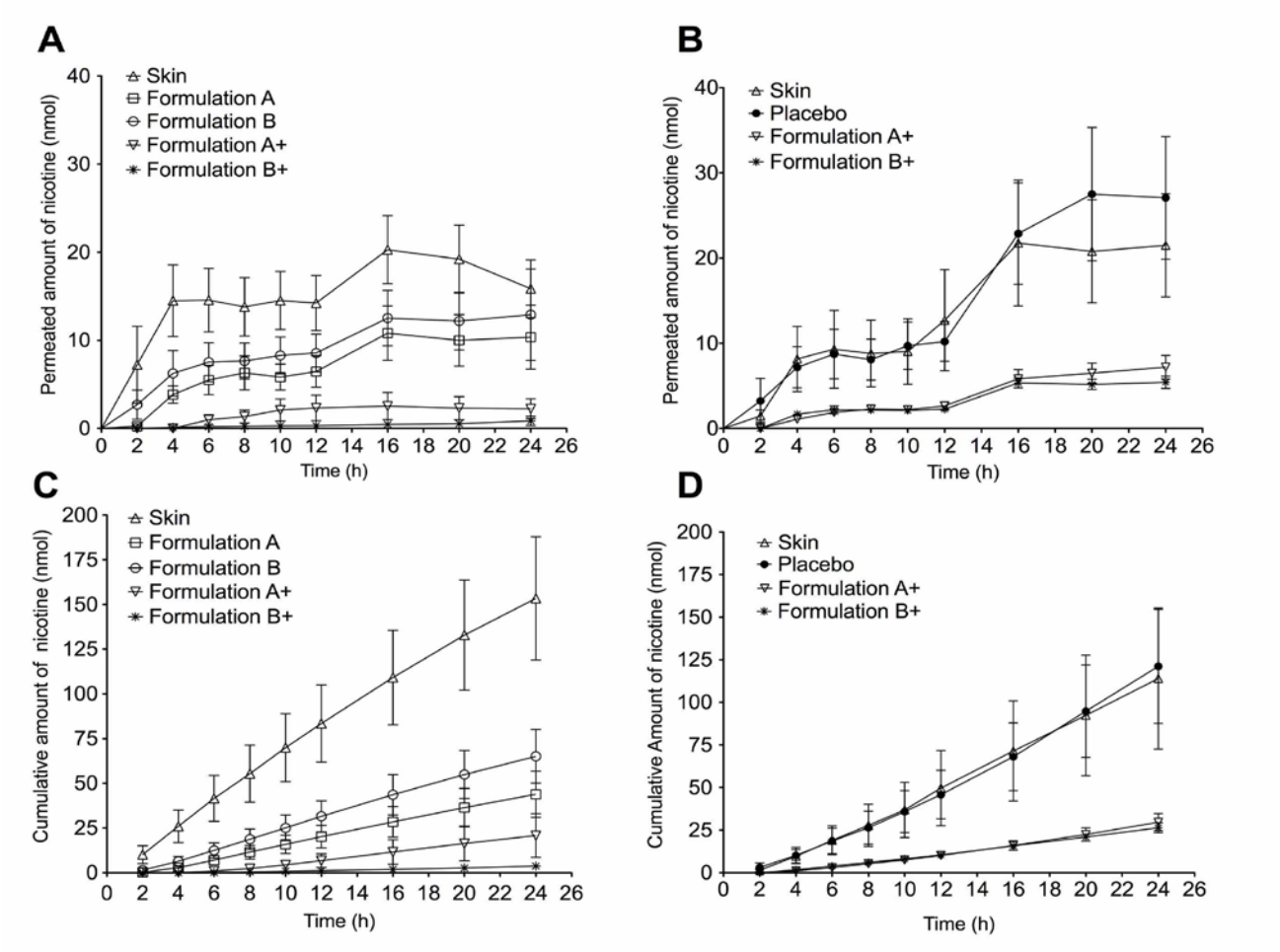

Figure 2. (A) Actual permeated amount of nicotine (nmol) versus time (h) for L-nicotine (donor solution 0.7 mg/mL); (B) actual permeated amount of nicotine (nmol) versus time (h) for nicotine (from green tobacco leaf extract); (C) cumulative permeation (nmol) of nicotine (from 0.7 mg/mL L-nicotine) through Yucatan minipig skin of untreated and Formulations A, B, A+ and B+ treated skin and (D) cumulative permeation (nmol) of nicotine from green tobacco leaf extract through Yucatan minipig skin of untreated and Formulations A+, B+ and placebo treated skin.

2) Permeation of nicotine from tobacco leaf extract:

Formulations A+ and B+ showed the highest percent reduction in the cumulative amount of nicotine over 24 h across Yucatan minipig skin, therefore they were selected for studies with the green tobacco leaf extract. The use of green tobacco leaf extract allowed for simulation of a slightly exaggerated exposure amount that tobacco farmers and farm workers may encounter, assuming the barrier cream may protect workers more effectively when utilized in the field. Both Formulation A+ and Formulation B+ resulted in a significant (p<0.05) reduction in nicotine cumulative permeation at 24 h (A+=29.66 ± 15.15 nmol and B+=26.40 ± 8.27 nmol) compared to untreated skin (73.24 ± 20.25 nmol) and placebo formulation treated skin (95.64 ± 55.87 nmol) (Table 1). Lag time for cumulative permeation of nicotine from tobacco extract through untreated skin was 5.0 ± 0.8 h; lag times for Formulations A+ and B+ were 5.5 ± 1.1 h and 4.3 ± 2.0 h, respectively (Table 1). Formulations A+ and B+ resulted in a 59.5% and 64.0% reduction in nicotine cumulative amount over 24 h, respectively, when tested in the *in vitro* diffusion apparatus with green tobacco leaf extract as the donor solution compared to untreated skin. The placebo formulation resulted in negative or no reduction (-30% which was assumed as 0% reduction) in nicotine from green tobacco leaf extract. The two formulations (A+ and B+) were determined to be ideal for the field testing with tobacco farmers and farm workers.

3) Permeation of nicotine from tobacco leaf extract through cotton gloves:
We further evaluated the use of coating Formulation B+ on gardening gloves. Brown gloves containing Formulation B+, control brown gloves and white gloves with Formulation B+ showed a significant decrease (p<0.05, one-way ANOVA and Tukey post-hoc analysis) in nicotine permeation (12.56 ± 3.62, 26.99 ± 4.53

and 26.07 ± 10.95 nmol, respectively) (Figure 3, Table 2) in the cumulative amount of nicotine at 24 h, compared to untreated skin (93.45 ± 27.24 nmol). Lag time for cumulative permeation of nicotine through untreated skin was 1.5 ± 0.8 h; lag times for brown gloves containing Formulation B+, control brown gloves, white gloves with Formulation B+ and control white gloves were 2.4 ± 1.6 h, 5.0 ± 1.5 h, 4.0 ± 2.3 h and 2.5 ± 1.7 h, respectively (Table 2). Lag times were not significantly different compared to the untreated skin. The percent reduction for brown gloves containing Formulation B+, control brown gloves and white gloves with Formulation B+ and control white gloves were calculated to be 86.6%, 71.1%, 72.1% and 41.8%, respectively, compared to untreated skin. However, brown gloves coated with formulation did not show a significant difference compared to plain brown gloves, even though the cumulative amount and percent reduction of permeated nicotine over 24 h was lower in comparison. Control white gloves did not show a significant decrease in permeation (54.41 ± 20.28 nmol) compared to untreated skin, where the percent reduction of cumulative amount at 24 h was 41.8%.

Table 2 Barrier cream formulations tested against L-nicotine and green tobacco leaf extract

Nicotine from green tobacco leaf extract as donor solution

Applied formulations	Cumulative amount at 24 h (nmol) Mean ± SD	Lag time (h) Mean ± SD	Percent reduction of cumulative amount at 24 h
Brown gloves (wB+) (n=8)	*12.56 ± 3.26	2.4 ± 1.6	86.6 %
Brown gloves (n=8)	*26.99 ± 4.53	5.0 ± 1.5	71.1 %
White gloves (wB+) (n=9)	*26.07 ± 10.95	4.0 ± 2.3	72.1 %
White gloves (n=5)	54.41 ± 20.28	2.5 ± 1.7	41.8 %
Untreated skin (n=8)	73.24 ± 20.25	1.5 ± 0.8	--

*Designates significant difference ($p<0.05$) compared to respective untreated skin values

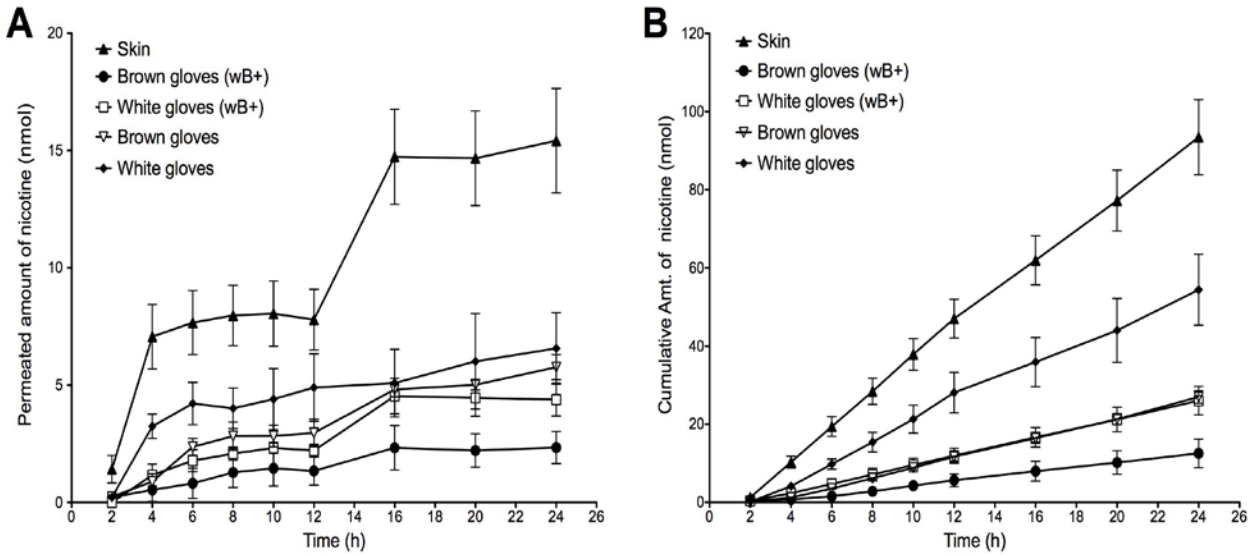

Figure 1. (A) Actual permeated amount of nicotine (nmol) versus time (h) for nicotine from green tobacco leaf extract; (B) Cumulative permeation (nmol) of nicotine from green tobacco leaf extract through Yucatan minipig skin. Skin samples inside the diffusion chambers were protected with a square section from gloves either treated or untreated with formulation B+ where wB+ signifies treated gloves.

Since A+ and B+ were the most effective formulations from *in vitro* studies, these were recommended for use in the field testing with tobacco farmers and farm workers.

Aim 3: To evaluate its efficacy, acceptance, side effects and feasibility in a field pilot study.

Methods:

1) Approval of the study by Food and Drug Administration (FDA) and Institutional Review Board (IRB):

The barrier cream was approved by FDA as an investigational new drug (IND) and this pilot field study was considered by FDA as a Phase I clinical trial. Application materials such as drug name, ingredients, potential toxicity of the ingredients and preparation methods were sent to FDA for review; conference call was made with FDA officials to discuss the details of the protocol. FDA requirements such as appropriate bottling, storage, labeling and use instructions were followed and adverse events during use were reported in time to FDA. After FDA approval, approvals from the IRB at UNTHSC and the University of Kentucky were obtained.

2) Recruitment of farmers and farm workers:

Co-I Dr. Robert Pearce and Linguistic Consultant Victoria Davis worked to recruit tobacco farms in Lexington, KY area. Dr. Pearce is the Agricultural Extension Specialist and has wide connection with tobacco growers. The farms of medium size that were willing to participate were recruited. The farms were recruited and studied on a rotary basis. All workers in the recruited farms were eligible to participate in the study but only those who were aged 21-65, willing to participate in the study and a non- smoker, had no history of liver, skin and cardiovascular diseases or for women not currently in pregnancy were consented. Consent procedures were followed. Women and minorities were actively recruited but no children <21 were recruited based on recommendations from FDA. The total number of workers was limited to 40.

3) Evaluation schedule:

Each farm was studied for 6 days. Prior to the study, the consent process was conducted. In each farm, recruited workers were randomly allocated into one of the two groups to receive one of the two barrier cream formulations, namely, A+ or B+. The first 3 days were observed as baseline without the use of a barrier cream formulation; the second 3 days were the intervention period in which one of the 2 formulations was given to the worker for use. Each formulation group consisted of 20 workers.

4) Urine collection to measure nicotine and cotinine:

The original plan was to collect 24 hour urine samples using a urine collection container. However, that was considered by the IRBs as not feasible. Instead, they recommended taking spot urine samples. Therefore, we collected pre-shift urine samples before the workers heading to tobacco fields and the post-shift urine samples after they finished the harvesting work and got back to their living places. A total of 12 samples (6 at baseline and 6 during cream use) were collected from each worker. Twelve 4 oz. Medline basic specimen container (cup) with screw-on lid, ID label and graduations in oz. and cc. and a urine collection instruction page in a plastic bag were given to each worker after the consent process. The workers left their urine in cups in their restroom and the study team members collected urine samples twice a day and placed them in a cooler with ice packs. The samples were transported to the laboratory at the University of Kentucky, College of Public Health where they were processed promptly. The total volume of each sample was recorded. Each sample was then poured into 1 Falcon conical centrifuge tube of 15 mL size and another tube of 50 mL size. The rest of the urine sample was discarded. The 50 mL tube was shipped to the PI's laboratory in Texas for storage in a freezer at -80^{o} C for backup use.

5) Urine sample analysis for nicotine and cotinine:

The 15 ml test tube was shipped in a cold container to the National Medical Services (NMS) Lab (Willow Grove, PA, http://www.nmslabs.com/) for analysis. The analysis was performed with their standard method Nicotine and Metabolite with Anabasine, Urine Test (9404U) for the measurement of urinary nicotine and cotinine and the method (1348U) for urinary creatinine using High Performance Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) and protocols for high quality controls. Due to the budget limitations, we were only able to analyze the post-shift urine samples.

6) Weight and height measurement for adjustment for body surface area:

The body surface area (BSA) is known to correlate with the half-life and metabolism of chemical substances and commonly used for adjusting their internal dosage. The urinary cotinine for each subject was adjusted for his/her BSA based on the method of the Mosteller formula: BSA (m^2) = $[(weight*height)/3600]^{1/2}$, which is about

1.7 m^2 for an adult. Weight was measured with a scale and height was measured with a tape measure. The urinary nicotine and cotinine concentrations were expressed in ng/ml/m^2. A cotinine level of 80 ng/ml/m^2 or above will be considered to reflect a positive absorption of nicotine, a higher value than passive smoking effects.

7) Questionnaire survey and subjective evaluation of the product formulations:

A questionnaire-type of form was used to collect data about the participants and evaluate the product formulation subjectively. The content of the questionnaire included 1) demographics such as age, gender, ethnicity, race, education, social-economic status, occupation, cigarette smoking and alcohol consumption; 2) a history of occupational exposure to nicotine from tobacco farm work; 3) health status, pregnancy status and any existing disorders, diseases and symptoms related to GTS; and 4) subjective evaluation on the product formulation. The questionnaire was self-administered with the help of the study team members or fellow workers. A 10-point scale was used for subjective evaluation on the product formulation which was conducted at the end of the study when the barrier cream formulations were used. Workers were asked about the efficacy, acceptability, side effects and ease of use of the formulations using the 10-point scale with 0 as the optimal score for side effects and 10 for others.

8) Collection on daily work tasks:

A daily work activity form was used to record the main tasks performed during the day such as the tasks handling green tobacco products and those not related to green tobacco products. The form was self-administered at the end of the work shift with the assistance of study team members or fellow workers. The daily activity form was also used to collect daily symptoms related to nicotine poisoning (all 6 days) and any symptoms related to the use (3 days) of the barrier cream formulation (side effects).

9) Worker safety, medical monitoring and adverse events:

A data safety monitor board (DSMB) was formed with a physician, a biostatistician and a physiologist from the UNTHSC that regularly met and provided the study team with guidance and recommendations. Adverse events were appropriately categorized and reported to FDA, CDC/NIOSH and IRBs promptly. Co-I Dr. Prince worked as a medical consultant and he and his occupational and environmental medicine resident were on call during the study. When adverse event symptoms were reported by a worker either related to the use of barrier cream or nicotine poisoning, he went to the site to examine the worker and provide medical advice for the worker and the team. Vital signs (blood pressure, heart rate and body temperature) were measured of each worker at baseline (prior to the study), during the 3 days with no cream use, during the cream use and at the end of the study.

10) Data analysis:

(1) Concentrations of nicotine and cotinine in the urine adjusted for urinary creatinine (μg/g) were calculated. (2) Worker characteristics were tabulated and analysis of variance (ANOVA) or two-sample t test was used to examine levels of urinary nicotine and cotinine by worker characteristics. (3) Mixed effects models with random effects for subjects were fitted to identify significant determinants of nicotine and cotinine levels. (4) A two-sample t test was used to compare the difference in levels of nicotine and cotinine in urine before and after the use of a barrier cream formulation to evaluate the efficacy of the two formulations. Vital signs were also analyzed among the four phases and specific cases of adverse events or nicotine poisoning were also described.

Results:

1) Worker characteristics:

A total of 43 workers from 6 farms were recruited with 40 males and 4 females, all of Hispanic origin. Mean age was 30.1 years. Seventeen of the workers (40%) had no education and the rest had 8 or more years of

education. Twenty two workers (51%) had one year of less experience working in the tobacco harvesting work. See Table 3 for details.

Table 3. Worker characteristics

Gender	Male	N=40	93%
	Female	N=3	7%
Race	Hispanic	N=43	100%
	Others	N=0	
Age (years)	Mean	30.1	
	SD	9.3	
Height (cm)	Mean	165.1	
	SD	6.8	
Weight (lbs.)	Mean	158.9	
	SD	23.3	
Education (years)	0	N=17	40%
	8	N=18	42%
	>8	N=8	18%
Years of work in tobacco harvesting	<=1	N=22	51%
	>1	N=21	49%

2) Urinary levels of nicotine and cotinine (adjusted by urinary creatinine level) by cream use and worker characteristics:

Table 4 shows the results of urinary nicotine and cotinine levels (adjusted by urinary creatinine level) by cream use and worker characteristics. Geometric mean (GM) ± geometric standard deviation (GSD) was significantly different between male and female workers with 507.2 (±0.002) vs 221.7 (±0.002) mg/g creatinine, $p<0.05$, for nicotine, and 297.4 (± 0.002) vs 96.2 (± 0.002) mg/g creatinine, $p<0.05$, for cotinine. GM (± GSD) was also significantly different between workers without education and those with1 to 8 years of schooling [370.4 (± 0.002) vs 598.0 (± 0.002) mg/g creatinine, $p<0.05$ for nicotine.

Table 4 also shows that urinary nicotine and cotinine levels (mg/g creatinine) were significantly higher when the barrier cream was used than those when no barrier cream was used with 485.9 (± 0.002) vs 408.6 (± 0.002) for nicotine ($p<0.001$) and 277.3 (± 0.002) vs 250.3 (± 0.002) for cotinine ($p<0.01$). No significant difference ($P>0.05$) in nicotine and cotinine levels was identified between the two groups who used different formulations (A+ vs. B+).

There was no significant difference in nicotine and cotinine levels for other worker characteristics.

Table 5 shows that only the use of a barrier cream was identified as the significant predictor of nicotine (mg/g creatinine, $p<0.01$) and cotinine (mg/g creatinine, $p<0.05$) in mixed-effect model analysis. No other factors were found as significant predictors.

Table 4. Urinary levels of nicotine and cotinine by worker characteristics

Characteristics			Nicotine adjusted for creatinine (µg/g)			Cotinine adjusted for creatinine (µg/g)		
		N	GM	GSD	P-value	GM	GSD	P-value
Gender	Male	40	507182.0	1.8		297366.0	1.8	
	Female	3	221699.1	1.7	0.02	96258.4	1.8	0.01
Race	Hispanic (All)	43	478728.6	1.8	NA	274862.5	1.9	
Age	<=30	26	515731.0	1.9	0.31	315820.1	2.0	0.05
	>30	17	427206.3	1.7		222256.8	1.7	
Education	0	17	370441.7	2.1		223816.7	2.2	
	<=8	18	597992.8	1.6	0.03	310975.5	1.6	0.17
	>8	8	500478.0	1.6	0.43	322174.3	1.8	0.23
Occupation	Local	3	326456.5	3.4		173741.5	3.6	
	Seasonal	30	525349.9	1.6	0.31	320867.6	1.7	0.22
	Migrant farm worker	10	406341.3	2.1	0.65	198265.9	2.0	0.82
Years of work in tobacco harvesting	<1	22	481628.3	1.9		310793.5	1.9	
	>=1	21	475709.6	1.8	0.85	241667.5	1.8	0.25
PPE use	None	7	413654.7	1.8		260550.8	2.0	
	Glove + shirt	4	304617.7	1.3	0.20	126363.1	1.6	0.03
	Shirt	32	523011.5	1.9	0.41	306466.2	1.8	0.81
Cream use	No	43	408553.4	2.0		250281.1	2.1	
	Yes	43	485947.7	2.1	0.01	277295.6	2.0	0.04

Table 5. Mixed effect model analysis on determinants of urinary nicotine and cotinine levels

Characteristics		Nicotine adjusted for creatinine (µg/g)				Cotinine adjusted for creatinine (µg/g)			
		Point estimate	P-value	Standard error	T-value	Point estimate	P-value	Standard error	T-value
Gender		-0.5	0.28	0.5	-1.1	-0.6	0.24	0.5	-1.2
Age		0.0	0.88	0.2	0.2	0.2	0.39	0.2	0.9
Education	<=8	0.4	0.10	0.2	2.0	0.2	0.50	0.2	0.7
	>8	0.4	0.20	0.3	0.8	0.4	0.21	0.3	1.3
Occupation	Migrant workers	0.4	0.26	0.4	1.1	0.4	0.28	0.4	1.1
	Others	0.4	0.32	0.4	1.0	0.4	0.36	0.4	0.9
Years of tobacco harvesting work		0.4		0.2	1.6	0.1	0.77	0.2	0.3
PPE use	1,2	-0.4	0.35	0.5	-0.9	-0.7	0.14	0.5	-1.5
	2	0.2	0.47	0.3	0.7	0.0	0.99	0.3	0.0
Cream use		0.3	0.01	0.1	2.8	0.2	0.04	0.1	2.1

3) Urinary levels of cotinine (adjusted by body surface area: µg/mL/m^3) by cream use and worker characteristics:

Table 6 shows the results of urinary nicotine and cotinine levels (adjusted by body surface area: µg/mL/m^3) by cream use and worker characteristics. Geometric mean (GM) ± geometric standard deviation (GSD) was significantly different between ages <=30 and >30 with 238.1 (± 1.8) vs 180.1 (± 1.5), $p<0.05$, for cotinine. There was no difference for nicotine. GM (± GSD) was also significantly different between workers without education and those with1 to 8 years of schooling [325.6 (± 1.9) vs 530.3 (± 1.5), $p<0.05$ for nicotine.

Table 6 also shows that urinary nicotine and cotinine levels (adjusted by body surface area: µg/mL/m^3) were significantly higher when the barrier cream was used than those when no barrier cream was used with 439.2 (± 2.0) vs 340.5 (± 2.0) for nicotine ($p<0.001$) and 230.1 (± 1.8) vs 183.7 (± 1.7) for cotinine ($p<0.01$). No significant difference ($P>0.05$) in nicotine and cotinine levels (adjusted by body surface area: µg/mL/m^3) was identified between the two groups who used different formulations (A+ vs. B+).

There was no significant difference in nicotine and cotinine levels (adjusted by body surface area: µg/mL/m^3) for other worker characteristics.

Table 7 shows that only the use of a barrier cream was identified as the significant predictor of nicotine (µg/mL/m^3, $p<0.01$) and cotinine (µg/mL/m^3, $p<0.05$) in mixed-effect model analysis. No other factors were found as significant predictors.

Table 6. Urinary levels of nicotine and cotinine by worker characteristics

Characteristics			Nicotine adjusted for body surface area (μg/mL/m^2)			Cotinine adjusted for body surface area (μg/mL/m^2))		
		N	GM	GSD	P-value	GM	GSD	P-value
Gender	Male	40	432.1	1.8		221.4	1.6	
	Female	3	302.6	1.8	0.37	129.4	2.0	0.14
Race	Hispanic (All)	43	421.5	1.8	NA	213.2	1.7	
Age	<=30	26	444.9	1.8	0.43	238.1	1.8	0.0467
	>30	17	387.9	1.8		180.1	1.5	
Education	0	17	325.6	1.9		182.5	1.8	
	<=8	18	530.3	1.5	0.02	238.4	1.5	0.12
	>8	8	435.1	1.7	0.73	230.8	1.8	0.36
Occupation	Local	3	317.2	2.3		136.0	2.1	
	Seasonal	30	437.3	1.7	0.28	235.2	1.7	0.16
	Migrant farm worker	10	410.9	1.8	0.30	181.8	1.6	0.39
Years of work in tobacco harvesting	<1	22	407.9	1.8		229.1	1.8	
	>=1	21	436.2	1.7	0.43	197.8	1.6	0.43
PPE use	None	7	328.0	1.6		198.1	1.7	
	Glove + shirt	4	349.4	1.7	0.63	128.6	1.7	0.12
	Shirt	32	455.8	1.8	0.34	230.8	1.6	0.77
Cream use	No	43	340.5	2.0		183.7	1.7	
	Yes	43	439.2	2.0	0.0028	230.1	1.8	0.003

Table 7. Mixed effect model analysis on determinants of urinary nicotine and cotinine levels

Characteristics		Nicotine adjusted for body surface area (μg/mL/m^2)				Cotinine adjusted for body surface area (μg/mL/m^2)			
		Point estimate	P-value	Standard error	T-value	Point estimate	P-value	Standard error	T-value
Gender		-0.0	0.95	0.5	-0.1	-0.1	0.84	0.4	-0.2
Age		0.1	0.75	0.2	0.3	0.2	0.25	0.2	1.2
Education	<=8	0.4	0.06	0.2	2.0	0.2	0.38	0.2	0.9
	>8	0.2	0.42	0.3	0.8	0.2	0.42	0.3	0.8
Occupation	Migrant workers	0.4	0.22	0.4	1.2	0.4	0.21	0.3	1.3
	Others	0.5	0.21	0.4	1.3	0.5	0.77	0.4	1.3
Years of tobacco harvesting work		0.4	0.1	0.2	1.7	0.1	0.77	0.2	0.3
PPE use	1,2	-0.2	0.59	0.4	-0.5	-0.5	0.24	0.4	-1.2
	2	0.3	0.28	0.3	1.1	0.1	0.75	0.3	0.3
Cream use		0.3	0.00	0.1	3.1	0.2	0.0003	0.1	3.7

4) Urinary levels of nicotine and cotinine by task:

Each worker performed 4 major tasks every day, namely, cutting tobacco stalks, pulling suckers off, loading tobacco stalks and leafs on trucks hanging tobacco stalks and leafs in barns. Workers on average spent 4.5 hours for cutting tobacco stalks, 0.5 hours for pulling suckers off, 0.2 hours for loading them on trucks and 3.3 hours for hanging them in barns.

Nicotine exposure (μg/g creatinine) was significantly ($p<0.05$) higher in hanging in the barns task (GM±GSD: 561100.4±1.5) than cutting stalks (332995.3±1.5) and pulling suckers off tasks (301751.4±1.5). No significant difference was observed for cotinine adjusted for creatinine or nicotine and cotinine adjusted for body surface area. See Figure 4.

5) Subjective evaluation on efficacy, acceptability, side effects and ease of use of the formulations:

Workers rated the barrier cream product as acceptable to use, no side effects and easy to use. Most workers also evaluated the barrier cream as effective in reducing nicotine exposure.

6) Vital signs during the study periods:

No significant changes were observed during different phases of the study. The vital signs were in normal ranges. See Figure 5 for more details.

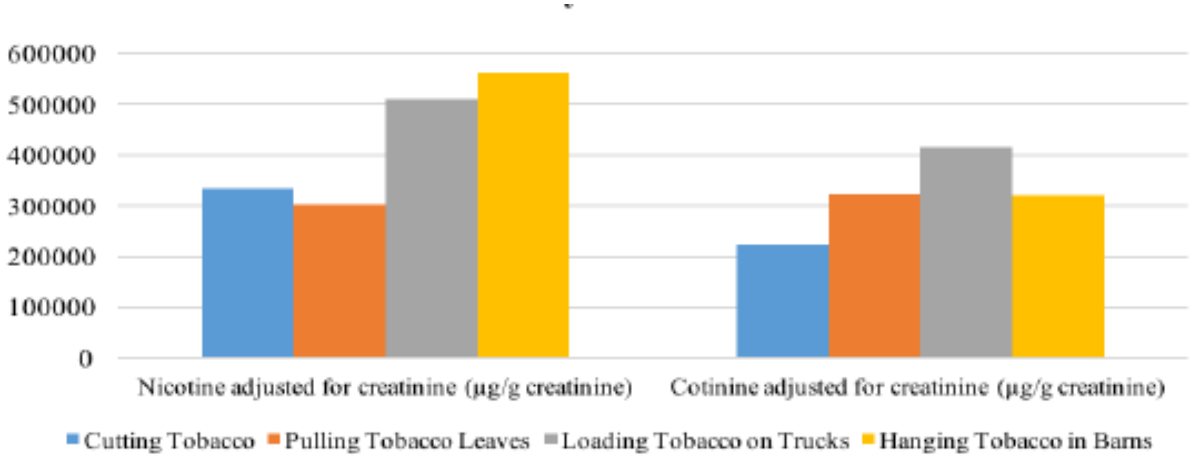

Figure 4. Nicotine and cotinine levels adjusted for urinary creatinine.

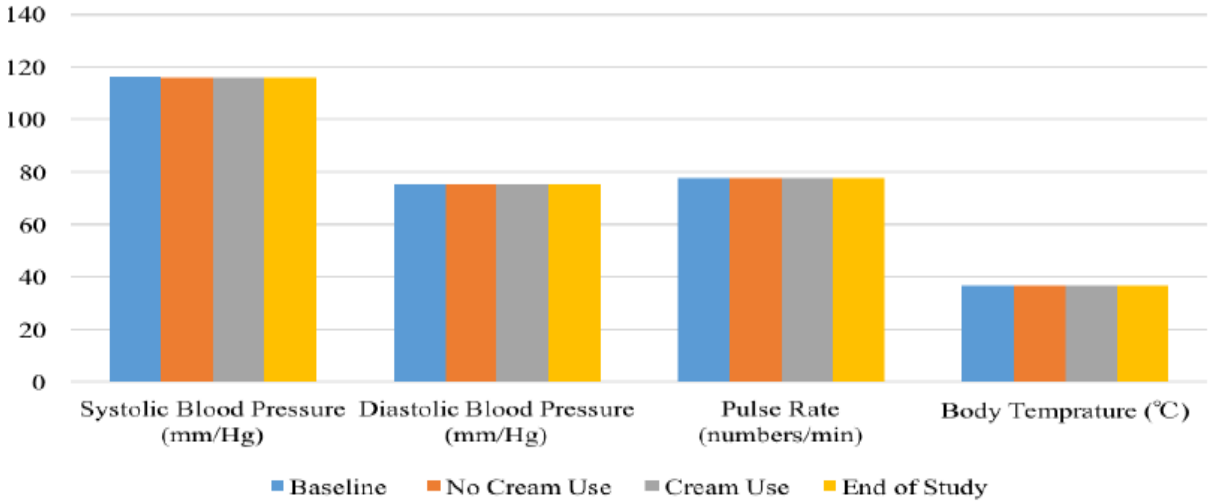

Figure 5. Vital sign measurements of workers by study phase.

7) Adverse events:

Table 8. Skin irritation and nicotine poisoning in tobacco harvesting workers.

Type of symptoms		Worker 1	Worker 2	Worker 3	Worker 4	Worker 5
Vomiting		+				+
Headache		+				+
Dizziness		+				+
Itchiness			+	+	+	
Blood pressure (mmHg)	Systolic	120	110	110	120	120
	Diastolic	70	70	70	90	70
Heart rate (numbers/min)		74	84	78	75	83
Temperature (°C)		36.9	36.9	37.2	36.8	36.4

Table 8 above shows 3 of the workers (Workers 2 to 4) had mild skin itchiness after the barrier cream formulation was applied, but no other symptoms. These workers were immediately instructed to stop using the formulation and examined by Co-I Dr. Prince for vital sign measurement and wellbeing. Dr. Prince did not find anything abnormal on the skin or with vital signs. The DMSB was consulted and they recommended the workers be allowed to remain in the study. Two workers also reported nicotine poisoning symptoms (vomiting, headache and dizziness). One occurred before the barrier cream use and the other during the cream use. Again, Dr. Prince examined the workers and found the symptoms were resolved by the end of the day. Their vital signs were in the normal range and therefore no treatment was provided.

Discussion:

1) *In vitro* study:

In addition to protective gear or even as a substitute, barrier creams may act as a second or third line of defense to prevent penetration of nicotine through the layers of the skin and finally into the vasculature. Barrier creams often work as skin protectants, which are intended for workers and employees to use prior to working with potentially harmful environmental chemicals. The effectiveness of barrier cream protectant formulations creates a physical diffusion barrier, and the acidic nature of the cream may influence a change in the chemical properties of the skin permeable toxic materials rendering them less harmful. Barrier cream formulations mentioned in this study were prepared from naturally occurring organic acids. Nicotine, when combined with an organic acid, for example tartaric acid, citric acid or ascorbic acid, forms a salt highly water soluble and less penetrable through the skin. Thus by changing the properties of nicotine base into a salt when it comes in contact with the applied barrier cream, the nicotine absorption is impeded at the skin surface.

In this *in vitro* testing, four different tested formulations of a barrier cream have been shown effective in reducing the amount of permeated nicotine through skin. The data indicates application of a barrier cream to the skin surface may significantly reduce the permeation of nicotine through skin when exposed to nicotine for 24 h. Of the tested barrier creams, Formulations A+ and B+ were the most effective of the creams, and were able to block 59.5% and 64.0% of the cumulative amount of nicotine absorption from green tobacco leaf extract when compared to the cumulative amount measured from untreated skin, respectively. These two formulations were also effective in reducing the absorption of L-nicotine by 86% to 98% when compared to permeation across untreated skin. This provided the basis that these two formulations be used in the field with tobacco farmers and farm workers. However, such methods may have some potential difficulty in implementing it effectively in the field. Therefore, it may be useful to incorporate formulations into clothing or gloves so workers could replace gloves when done. This may further reduce exposure to nicotine. Out of the tested formulations, Formulation B+ was chosen as the best fit considering it had the best performance when comparing percent reduction in nicotine concentration. Two different types of gardening gloves (brown thick gardening gloves and white thinner material gardening gloves) were selected and coated with Formulation B+. The effectiveness of these coated gloves was tested against green tobacco leaf extract. The coated gloves showed an 86.6% (brown glove) and 72.1% (white glove) reduction in permeation when compared to untreated skin (no gloves) suggesting the formulation is stable and performs well when incorporated with an existing commercial product. The coated glove has a potential for future field use, but more testing is needed.

2) Field pilot study with migrant tobacco farm workers:

In this field study, the efficacy was not optimal as we expected. Instead of reducing the nicotine and cotinine levels during the use, the levels were actually increased after the use of the barrier cream formulations. We were perplexed by the results as the *in vitro* testing showed good percentage of reduction in both nicotine solution and tobacco extract. Possible reasons are as follows: (1) the tasks performed in the three days before the cream use and the 3 days during the cream use were not the same or standardized. Therefore, it is possible the workers were performing tasks with lower exposures during the first 3 days and performing tasks that had much more exposure such as hanging tobacco stalks and leafs in barns during the cream use; (2) limitation due to the fact the end of work shift urine samples were analyzed and used without the analysis and use of pre-shift samples as a comparison. Cross-shift difference of urinary nicotine and cotinine might be a better indicator of the efficacy; (3) possible human errors in the laboratory preparation of the formulations with inadequate quantities of acids; (4) the application process was not supervised on site: the cream bottles were given to the workers at prior night and workers were instructed to apply to exposed areas of the body; they

were not supervised for the application. It is possible the workers might not have actually used them due to the suspicion on its toxicity, or even they used them, they did not use enough amount to cover the whole exposed area. Additionally, reapplication was needed, but workers might not have done that; 5) small sample size. Further testing with large sample and to correct the above possible limitations is needed.

The subjective evaluations from workers were good about the acceptability, ease to use and potential side effects. Workers also made some suggestions to improve the formulation such as adding some fragrance. Further testing would need to take into account of these suggestions and subjective evaluations.

The cream does cause mild skin irritation to a small number of workers who are more sensitive to the acidic ingredients. However, no abnormal and objective skin reactions were identified upon examination. Further testing is also needed to observe its side effects more.

Our pilot study also revealed that workers do get GTS symptoms when perfuming the harvesting tasks. Since about 50% of the workers were first time harvesting tobacco, they might not be experienced and well trained in nicotine exposure and GTS prevention. It is necessary to conduct a study to find out which methods in addition to the use of a barrier cream could help workers particularly new workers increase their knowledge, change their attitude and behaviors (work practices) and reduce GTS symptoms.

Conclusions and recommendations:

We developed four formulations of a barrier cream for reducing skin permeation of nicotine and tested them in both in vitro studies and in the field with migrant tobacco harvesters. While in vitro testing showed significant reduction of the formulations. The results from the field testing were not optimal and as expected due to possible limitations of the study. Further studies of the cream with large sample size of farms and workers, better design of the tasks performed for comparison and improved application of the cream on exposed skin areas are needed. Additionally, an intervention study with comprehensive skin exposure prevention program is needed to identify effective components of the intervention program is needed so that effective and feasible measures can be recommended to these workers to reduce nicotine exposure and GTS.

PUBLICATIONS

1. Liu Y, Pearce B, Okafor C, Gohil V, Prince TS, Biswas S, Reed D, Davis VG, Stinchcomb A, Klingner T, Sterling D. Efficacy of a barrier cream in reducing dermal exposure to nicotine from green tobacco harvesting work: a pilot study. To be submitted to American Journal of Industrial Medicine.
2. Andar A, Liu Y, Sterling D, Klingner T, Tokarski M, Boeniger M, Hammell D, Stinchcomb A. Barrier cream formulations to reduce skin permeation of nicotine as a preventive measure for green tobacco sickness. Presented as a poster at the American Association of Pharmaceutical Scientists Annual Meeting and Exposition. Occupational and Environmental Medicine (Submitted and under review).
3. Okafor C, Gohil V, Liu Y, Sterling DA, Pearce R, Biswas S, Prince TS, Davis G, Carol M, Reed D, Klingner T, Tokarski M, Stinchcomb A. Determinants of nicotine exposure in tobacco harvesting workers: a pilot study (Mentor for the Students Okafor C and Gohil V). Presented at the American Industrial Hygiene Conference and Exposition, Baltimore, MD, May 21 - 26, 2016.
4. Gohil V, Okafor C, Liu Y, Sterling DA, Pearce R, Prince TS, Davis G, Carol M, Reed D, Biswas S, Stinchcomb A. Tobacco harvesting work, exposure to nicotine, vital signs and nicotine poisoning (Mentor for the Students Gohil V and Okafor C). Presented at the American Industrial Hygiene Conference and Exposition, Baltimore, MD, May 21 - 26, 2016.
5. Andar A, Liu Y, Sterling D, Klingner T, Tokarski M, Boeniger M, Hammell D, Stinchcomb A. Barrier cream Formulations to reduce skin permeation of nicotine as a preventive measure for Green Tobacco Sickness. Presented as a poster at the American Association of Pharmaceutical Scientists Annual Meeting and Exposition. Presented as a poster at the American Association of Pharmaceutical Scientists Annual Meeting and Exposition. San Diego, CA, November 2 to 6, 2014.

AWARDS RECEIVED

1. American Industrial Hygiene Association (AIHA) Social concerns Committee 2016: Appreciation for a Significant Contribution to Technical Knowledge in Areas of Social Concern in the Field of Occupational Safety and Health for “Okafor C, Gohil V, Liu Y, Sterling DA, Pearce R, Biswas S, Prince TS, Davis G, Carol M, Reed D, Klingner T, Tokarski M, Stinchcomb A. Determinants of Nicotine Exposure in Tobacco Harvesting Workers: A Pilot Study (Mentor for the Students Okafor C and Gohil V)”. The award was mailed in August, 2016.

2. American Industrial Hygiene Association (AIHA) Social concerns Committee 2016: Appreciation for a Significant Contribution to Technical Knowledge in Areas of Social Concern in the Field of Occupational Safety and Health for “Gohil V, Okafor C, Liu Y, Sterling DA, Pearce R, Prince TS, Davis G, Carol M, Reed D, Biswas S, Stinchcomb A. Tobacco Harvesting Work, Exposure To Nicotine, Vital Signs And Nicotine Poisoning (Mentor for the Students Gohil V and Okafor C)”. The award was mailed in August, 2016.

3. American Industrial Hygiene Association (AIHA) Personal Protective Equipment Committee 2016: Best Student Poster Award for “Okafor C, Gohil V, Liu Y, Sterling DA, Pearce R, Biswas S, Prince TS, Davis G, Carol M, Reed D, Klingner T, Tokarski M, Stinchcomb A. Determinants of Nicotine Exposure in Tobacco Harvesting Workers: A Pilot Study (Mentor for the Students Okafor C and Gohil V)”. The award was presented at the American Industrial Hygiene Conference and Exposition, Baltimore, MD, May, 2016.

4. American Industrial Hygiene Association (AIHA) Biological Monitoring Committee 2016: Best Student Poster Award for “Gohil V, Okafor C, Liu Y, Sterling DA, Pearce R, Prince TS, Davis G, Carol M, Reed D, Biswas S, Stinchcomb A. Tobacco Harvesting Work, Exposure To Nicotine, Vital Signs And Nicotine Poisoning (Mentor for the Students Gohil V and Okafor C)”. The award was presented at the American Industrial Hygiene Conference and Exposition, Baltimore, MD, May, 2016.

INCLUSION OF GENDER AND MINORITY STUDY SUBJECTS

Both male (40) and female (3) workers were included in the study. All of them were migrant Hispanic workers from Mexico. Other ethnic workers were either not available or not willing to participate.

INCLUSION OF CHILDREN

No children under the age of 21 were included in the study per FDA requirement although children do work in this work setting. Therefore, some workers younger than 21 were not eligible to participate in the study.

APPENDIX

1) Manuscript on in vitro study: Andar A, Liu Y, Sterling D, Klingner T, Tokarski M, Boeniger M, Hammell D, Stinchcomb A. Barrier cream formulations to reduce skin permeation of nicotine as a preventive measure for green tobacco sickness. Presented as a poster at the American Association of Pharmaceutical Scientists Annual Meeting and Exposition. Occupational and Environmental Medicine (Submitted and under review).
2) Four award certificates:
    a. American Industrial Hygiene Association (AIHA) Social concerns Committee 2016: Appreciation for a Significant Contribution to Technical Knowledge in Areas of Social Concern in the Field of Occupational Safety and Health for “Okafor C, Gohil V, Liu Y, Sterling DA, Pearce R, Biswas S, Prince TS, Davis G, Carol M, Reed D, Klingner T, Tokarski M, Stinchcomb A. Determinants of Nicotine Exposure in Tobacco Harvesting Workers: A Pilot Study (Mentor for the Students Okafor C and Gohil V)”. The award was mailed in August, 2016.
    b. American Industrial Hygiene Association (AIHA) Social concerns Committee 2016: Appreciation for a Significant Contribution to Technical Knowledge in Areas of Social Concern in the Field of Occupational Safety and Health for “Gohil V, Okafor C, Liu Y, Sterling DA, Pearce R, Prince TS, Davis G, Carol M, Reed D, Biswas S, Stinchcomb A. Tobacco Harvesting Work, Exposure To Nicotine, Vital Signs And Nicotine Poisoning (Mentor for the Students Gohil V and Okafor C)”. The award was mailed in August, 2016.
    c. American Industrial Hygiene Association (AIHA) Personal Protective Equipment Committee 2016: Best Student Poster Award for “Okafor C, Gohil V, Liu Y, Sterling DA, Pearce R, Biswas S, Prince TS, Davis G, Carol M, Reed D, Klingner T, Tokarski M, Stinchcomb A. Determinants of Nicotine Exposure in Tobacco Harvesting Workers: A Pilot Study (Mentor for the Students Okafor C and Gohil V)”. The award was presented at the American Industrial Hygiene Conference and Exposition, Baltimore, MD, May, 2016.
    d. American Industrial Hygiene Association (AIHA) Biological Monitoring Committee 2016: Best Student Poster Award for “Gohil V, Okafor C, Liu Y, Sterling DA, Pearce R, Prince TS, Davis G, Carol M, Reed D, Biswas S, Stinchcomb A. Tobacco Harvesting Work, Exposure To Nicotine, Vital Signs And Nicotine Poisoning (Mentor for the Students Gohil V and Okafor C)”. The award was presented at the American Industrial Hygiene Conference and Exposition, Baltimore, MD, May, 2016.
3) Equipment inventory
4) Invention statement.

Barrier cream formulations to reduce skin permeation of nicotine as a preventive measure against green tobacco sickness

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WHAT THIS PAPER ADDS?

- Tobacco harvesting workers are highly exposed to nicotine that results in green tobacco sickness. Current personal protective methods to reduce nicotine exposure are not so feasible. We developed a barrier cream as an alternate method of protection.
- This study demonstrates that two barrier cream formulations significantly reduced nicotine permeation through the skin either from L-nicotine solution or from green tobacco extracts as a source.
- When coated on gloves, the barrier cream showed more reduction in nicotine permeation through the skin which demonstrates that the barrier cream can be used either directly on skin or in conjunction with existing protective clothing.
- Field use of this barrier cream can potentially reduce the occurrence of green tobacco sickness that is a significant occupational disease in tobacco farmers and farm workers.

ABSTRACT

Objectives: Tobacco harvesting workers may have high levels of skin exposure to nicotine that can lead to green tobacco sickness. Current exposure reduction methods are often infeasible. The purpose of this work was to evaluate the effectiveness of topical barrier cream formulations as a personal protective equipment to reduce nicotine permeation through skin.

Methods: Barrier cream applied on Yucatan minipig skin was tested using a PermeGear flow through *in vitro* diffusion apparatus, where donor solutions of either L-nicotine or green tobacco leaf extract from the receiver compartment collected every 2 h was analyzed over a 24 h exposure period using high pressure liquid chromatography.

Results: The best barrier cream formulations reduced *in vitro* skin permeation of nicotine by 97.6% from L-nicotine, by 64.0% from green tobacco leaf extract and by 86.6% from green tobacco leaf extract for gardening gloves coated with the barrier cream.

Conclusions: Barrier creams are effective in reducing skin permeation of nicotine and might have greater preventive capabilities at environmental exposure levels of nicotine during tobacco harvesting.

INTRODUCTION

Tobacco is grown in some states of the U.S. and around world. Tobacco farmers and farm workers can have green tobacco sickness (GTS) or nicotine poisoning through the contact with green tobacco leaves and leaf saps during cultivation and harvest [1]. GTS often occurs when tobacco harvesting workers cut tobacco plants in the field, load them onto trucks and hang them in barns. The onset of the illness is between three to seventeen hours after exposure and the duration of the illness is one to three days. Symptoms of GTS include nausea, vomiting, muscle weakness, dizziness and may sometimes cause fluctuations in blood pressure or heart rate [2]. GTS symptoms are similar to those induced by pesticide exposure, heat exhaustion, or nicotine intoxication experienced by novice smokers. Symptoms can be so severe as to require emergency room visit. It also causes significant discomfort resulting in lost productivity among tobacco harvesting workers [1,3].

The primary cause of GTS is skin exposure to dissolved nicotine contained in the dew or water droplets on the tobacco leaves. Green tobacco plants are often harvested in early mornings or after a rainfall when leaves are covered with excessive dew or moisture [2]. Nicotine has a low molecular weight (162.2 g/mol) and is highly soluble in both polar and non-polar solvents creating an effective skin penetrant [4,5]. Nicotine has been shown to permeate the skin at far greater rates from aqueous solutions than from nearly nicotine solutions [4]. Dew or rain soaked fields can be increasingly hazardous to tobacco workers [1,2,6,7]. Unfortunately, farmers opt to harvest in the early mornings to avoid the harsh sunlight or due to a busy harvesting schedule from the growers (farmers), in doing so they work under higher humidity conditions. The farmers and farm workers are more vulnerable to higher nicotine exposure on humid days, especially after a recent rainfall. The average farm worker may be exposed to up to 600 mL

(which may contain around 54 mg of nicotine) of residual tobacco leaf moisture, the nicotine equivalent of 6-9 cigarettes [2,6,7]. Absorbed nicotine affects the central nervous system, and a lethal dose can be as low as 40 to 60 mg [3]. However, it is not known how much nicotine may trigger symptoms of GTS, since the probability of getting GTS varies depending on prior exposure to nicotine, for example, and if the individual was a smoker or not. It is evident that non-smokers, new farm workers and children have a higher probability of getting GTS, and therefore are at greater risk [6]. It is therefore clear and evident that nicotine overexposure in the fields is a health hazard in the agricultural community that must be prevented.

While typical exposure control hierarchical measures are not widely used in this occupation, some methods are recommended to reduce nicotine exposure. These include waiting for the morning dews on tobacco leaves to dry before starting harvesting, use of protective, water-resistant clothing, such as rubberized waterproof suits and gloves, changing to dry clothes between harvesting shifts and education of workers and farmers on nicotine hazard and GTS. However, these methods may not be practical. Avoidance of working under wet conditions is virtually impossible as the harvest season is short and requires 10-12 h work days; therefore, the growers or farmers may not be willing to delay work until the plants are dry. While special personal protective equipment (PPE) have shown to provide workers the required protection, they are extremely uncomfortable, cumbersome, bulky and too warm at high temperatures, since tobacco is harvested during the summer and early fall season [2,6,7]. Therefore, the use of these PPE may cause heat stress in workers. Efforts have also been made to educate workers on proper tobacco plant handling and the harmful effects of nicotine dermal absorption. Education and work practice change of workers is difficult, particularly in migrant populations, which make up an increasing number of workers in the tobacco fields for a number of reasons [6,8]. These

populations often face language barriers, lack of healthcare access due to poverty or immigration status, and lack of transportation. Additionally, following guidelines associated with frequent hand washing or changing to dry clothes can only protect an individual from nicotine exposure to a certain degree, when one takes into consideration the practicalities of the working environment and unavailability of clean water and dry clothes.

The development of a topical formulation that binds to nicotine or changes the acidity of skin thus preventing environmental nicotine absorption would be a highly advantageous solution in preventing GTS [6], and thereby eliminate the difficulties faced with current prevention methods. Barrier creams have been used in the workplace to reduce skin absorption from chemicals such as metal working fluid [9], gasoline engine oil [10], and organophosphate compounds [11]. To date, however, no such barrier cream is available on the market that could be used by tobacco farm workers to aid in the reduction of nicotine absorption via skin exposure. We successfully developed several formulations of a nicotine barrier cream based on the principle that nicotine exists in tobacco as a lipophilic free base that can readily penetrate the skin whereas nicotine forms a water-soluble salt with almost any organic acid such as ascorbic acid or tartaric acid. A nicotine salt would less readily pass through the skin barrier and can easily be washed off the skin after work.

The purpose of this work was to demonstrate the effectiveness of different topical formulations of a barrier cream in reducing nicotine permeation through Yucatan minipig skin *in vitro*. The objectives were 1) to evaluate the effectiveness in reducing nicotine permeation using nicotine solution, 2) to evaluate the effectiveness in reducing nicotine permeation using green tobacco extract and 3) to evaluate the effectiveness in reducing nicotine permeation using gardening gloves coated with the barrier cream formulations. Our ultimate goal is to develop viable

formulations that can be used in the field by tobacco farmers and farm workers to reduce their skin exposure to nicotine and therefore the occurrence of GTS.

EXPERIMENTAL METHODS

Materials

Ammonium acetate, sodium chloride, methanol, and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ). L-Nicotine was obtained from Sigma (St. Louis, MO). Saline solution (0.9%) was purchased from Teknova (Hollister, CA). De-ionized water was generated from a Milli-Q system (EMD Millipore; Billerica, MA). Full thickness Yucatan minipig skin was purchased from Sinclair Bio Resources, LLC. (Columbia, MO). Flow-through In-Line cells were purchased from PermeGear (Hellertown, PA) for the skin diffusion studies. Gloves were purchased from Grainger Inc. (www.grainger.com)(Lake Forest, IL). Tobacco extract was provided by a laboratory at the University of Kentucky College of Agriculture.

Preparation of barrier cream formulations

For preparation of Formulation A, 1.5 % w/v of methylcellulose was added to purified hot water (80-90°C) followed by the addition of citric acid (0.5% w/v) and tartaric acid (0.5% w/v). A pH of about 1 to 2 was observed. Potassium acetate (0.25% w/v) was added to increase the pH to 3.0. Polysorbate 80 was added as a surfactant/emulsifier. Formulation A+ was a more acidic formulation compared to Formulation A, with 1.5 % w/v of citric acid and 1.5 % w/v of tartaric acid. Methylcellulose amount was unchanged at 1.5% w/v and potassium acetate was increased to 0.7% w/v.

For preparation of Formulation B, 3.0% w/v of methylcellulose was dissolved in purified hot water (80-90°C). To this, citric acid (0.7% w/v), ascorbic acid (0.9% w/v) and tartaric acid (0.6% w/v) were added. The solution remained clear and had a pH of approximately 3.0. Polysorbate

80 was added as a surfactant/emulsifier. Formulation B+ contained ascorbic acid and citric acid each increased to 3.0% w/v, 1.6% w/v of methylcellulose and 1.0% w/v of potassium acetate. Placebo formulation was prepared to better demonstrate the effectiveness of the barrier creams. This formulation was prepared with 0.8% w/v of methylcellulose and excluded the ascorbic or citric acid, with a pH of 7.0.

To begin testing the gloves for protective efficiency, each glove type was first cut into a square with (~4.84 cm^2) to fit into the chambers above the skin (Figure 2A). The glove section was placed between the skin and top donor chamber of the In-Line flow cell and clamped down. The setup was equilibrated for 15 min before beginning the experiment with the appropriate donor solution (tobacco extract).

Preparation of gloves coated with barrier cream

Two types of gardening gloves were tested (Figure 2), thick knitted cotton gloves (brown gloves) and thin cotton protective gloves (white gloves). Blended cotton gardening gloves were selected since cotton-polyester blended clothing is what farmers and farm workers conventionally wear during harvest in warm weather [7]. Both these gloves were immersed and soaked in Formulation B+ for 5 min and air dried prior to diffusion studies.

Brown gloves: Natural knit polyester/cotton work gloves, medium size, medium weight, light brown color, 7 gauge, (Grainger Inc.,www.grainger.com, Lake Forest, IL) (Figure 2A). The knit construction allows breathability with less hand fatigue. These gloves may be used for gardening, warehousing, parts handling, assembly and food processing.

White gloves: Inspector ambidextrous/hemmed cotton gloves, medium weight, white color, 7 gauge (Grainger Inc., www.grainger.com, Lake Forest, IL) (Figure 2B). These gloves can be used as protective liners for hands and ideal for light gardening and chores.

Both the brown and white gloves were immersed into a solution of Formulation B+ and soaked for 5 min before air drying them. After being air dried, they were stored in re-sealable bags until further use with diffusion experiments. Tests for protective efficiency of each glove type against tobacco extract was performed as previously described.

In vitro skin diffusion studies

The Yucatan minipig skin was dermatomed to a thickness of approximately 250 µm using a Padgett® dermatome and then stored at -20°C until used. Stored skin samples were thawed to room temperature at the time of the experiment. Prior to placing the skin section in the diffusion cell, 40 µL of the investigational barrier formulation was gently rubbed onto the surface using a clean Teflon stir bar. The barrier cream was gently rubbed into the skin (0.95 cm^2) using a circular motion for approximately 30 seconds and then allowed to dry for approximately 20–30 min. This amount and time allowed adequate formulation coverage of the exposed skin area without leaving an undesirable pooling of the investigational formulation in the well of the diffusion cell, nor excess on the skin which would be undesirable for users of the formulation in the translational environment.

The flow-through diffusion cell system with diffusional area of 0.95 cm^2 was used for all *in vitro* skin studies. Skin surface was maintained at 32°C with a circulating water bath. Receiver solution was saline (0.9% w/v of high pressure liquid chromatography [HPLC] grade sodium chloride [NaCl] in water). To test the gardening gloves, the square section of fabric was made to fit the entire diffusion cell to simulate complete cover/protection, as would be on the hand. Nicotine donor solutions consisted of L-nicotine in water at a concentration of 0.7 mg/mL or green leaf tobacco extract. Five hundred µL of donor solution (either 0.7 mg/mL of L-nicotine or green leaf tobacco extract, containing 0.1 ± 0.05 mg/mL of nicotine) was placed on top of

each pretreated skin section or the pretreated glove section. For control diffusion cells, just nicotine solution or green leaf tobacco extract was placed on the skin, without the barrier cream pretreatment.

Sample analysis

All nicotine samples were analyzed by high performance liquid chromatography (HPLC) consisting of a Waters (Milford, MA) Alliance Separations Module e2695 with column heater, a Waters 2489 dual absorbance detector set at a wavelength of 260 nm and Waters Empower™ software. Additionally, a Brownlee (Perkin Elmer®; Wellesley, MA) C-18 reversed-phase Spheri-5 µm column (220 x 4.6 mm) with a C-18 reversed phase 7 µm guard column (15 x 3.2 mm) was used. The mobile phase consisted of 30 mM ammonium acetate (with 2% acetonitrile):methanol (30:70) and flow rate of 1.0 mL/min. The injection volume was 100 µL, with a run time of 11 min and the retention time of nicotine was 5.8 min.

Diffusion samples were collected and analyzed by HPLC. Nicotine standards were prepared in 0.9% saline solution (0.9% w/v of HPLC grade NaCl in MilliQ filtered water). The limit of detection (LOD) (0.08 µg/mL) and limit of quantification (LOQ) (0.15 µg/mL) were determined. All samples and standards were injected in duplicate. Working standard solutions were prepared fresh daily in the range of 0.08–10 µg/mL. All samples analyzed were within the standard curve range.

Data analysis

The permeation data were plotted as cumulative amount of nicotine collected in the receiver compartment as a function of time. Lag time was determined by calculating the average of the x-intercepts upon extrapolation of the slope line from the cumulative values. The percent reduction of cumulative amount of nicotine was determined by using equation 1, where the

percent difference between cumulative amounts at 24 h for treated skin and untreated skin was calculated for each formulation.

$$\text{Percent Reduction Cumulative Amount} = \frac{Cm_{skin} - Cm_f}{Cm_{Skin}} \times 100 \quad (1)$$

Where Cm_{skin} is the cumulative amount of nicotine through skin at 24 h without treatment and Cm_f is the cumulative amount of nicotine at 24 h of skin treated with formulation (either by Formulation A, B, A+, B+, Formulation B+ treated brown gloves or Formulation B+ treated white gloves). Statistical significance for nicotine diffusion through the skin was determined by one-way analysis of variance (ANOVA) and Tukey post-hoc analysis where $p<0.05$ was selected using SigmaStat 3.5 software (San Jose, CA), to determine the significant differences between the tested barrier cream formulations and gloves.

Transepidermal water-loss (TEWL) measurements of the skin allowed for testing the integrity of the dermatomed skin (cyberDERM RG_1 Evaporimeter; Broomall, PA). TEWL values of <10 g/m^2h were regarded as acceptable values for the diffusion experiments.

RESULTS

Barrier cream formulations tested against nicotine permeation

L-nicotine

In this study we evaluated barrier cream Formulations A, B, A+ and B+. All barrier cream formulations showed a significant decrease ($p<0.05$) in nicotine permeation compared to untreated skin (Figure 1, Table 1). Formulations A, B, A+ and B+ showed a significant reduction in the cumulative amount of nicotine at 24 h (43.88 ± 34.17, 65.13 ± 42.33, 20.81 ± 24.47 and 3.74 ± 2.10 nmol, respectively) compared to untreated skin (153.41 ± 91.22 nmol) (Table 1). Formulations A+ and B+ were the most effective barriers for the L-nicotine (0.7 mg/mL) donor solution and were selected for additional testing with green tobacco leaf extract. Lag time for

cumulative permeation of nicotine through untreated skin was 0.9 ± 1.1 h; lag times for Formulations A, B, A+ and B+ were 1.8 ± 1.8 h, 1.3 ± 1.9 h, 7.0 ± 3.6 h and 7.7 ± 3.7 h, respectively (Table 1). Formulations A+ and B+ lag times were significantly longer (p<0.05), compared to the lag times of Formulation A, Formulation B and untreated skin. Formulation A and B lag times were not significantly different (p>0.05) compared to the untreated skin. The percent reductions in cumulative amounts at 24 h for Formulations A+ and B+ were considerably higher (86.4% and 97.6%, respectively) compared to Formulations A and B (71.4% and 57.5%, respectively) tested with the 0.7 mg/mL L-nicotine donor solution.

Table 1 Barrier cream formulations tested against 0.7 mg/mL L-nicotine and green tobacco leaf extract

0.7 mg/mL L-nicotine donor solution			
Applied formulations	**Cumulative amount at 24 h (nmol) Mean ± SD**	**Lag time (h) Mean ± SD**	**Percent reduction of cumulative amount at 24 h**
Formulation A (n=7)	*43.88 ± 34.17	1.8 ± 1.82	71.4 %
Formulation B (n=8)	*65.13 ± 42.33	1.3 ± 1.87	57.5 %
Formulation A+ (n=4)	*20.81 ± 24.47	*7.0 ± 3.56	86.4 %
Formulation B+ (n=3)	*3.74 ± 2.10	*7.7 ± 3.74	97.6 %
Untreated skin (n=7)	153.41 ± 91.22	0.9 ± 1.10	--
Green tobacco leaf extract donor solution			
Formulation A+ (n=9)	*29.66 ± 15.15	5.47 ± 1.13	59.5 %
Formulation B+ (n=8)	*26.40 ± 8.27	4.33 ± 2.02	64.0 %
Placebo (n=4)	95.64 ± 55.87	6.06 ± 1.67	0% (-30.6%)
Untreated skin (n=5)	73.24 ± 20.25	5.02 ± 0.78	--
*Designates significant difference (p<0.05) compared to respective untreated skin values			

Green tobacco leaf extract

Formulations A+ and B+ showed the highest percent reduction in the cumulative amount of nicotine over 24 h across Yucatan minipig skin; therefore, they were selected for additional studies using green tobacco leaf extract. The use of green tobacco leaf extract allowed for simulation of a slightly exaggerated exposure amount that tobacco farmers and workers may encounter, assuming the barrier cream may protect workers more effectively when utilized in the field. Both Formulation A+ and B+ resulted in a significant reduction (p<0.05) in nicotine

cumulative permeation at 24 h (A+=29.66 ± 15.15 nmol and B+=26.40 ± 8.27 nmol) compared to untreated skin (73.24 ± 20.25 nmol) and placebo formulation treated skin (95.64 ± 55.87 nmol) (Table 1). Lag time for cumulative permeation of nicotine from green tobacco leaf extract through untreated skin was 5.0 ± 0.8 h; lag times for Formulations A+ and B+ were 5.5 ± 1.1 h and 4.3 ± 2.0 h, respectively (Table 1). Formulations A+ and B+ resulted in a 59.5% and 64.0% reduction in nicotine cumulative amount over 24 h, respectively, when tested in the *in vitro* diffusion apparatus with green tobacco leaf extract as the donor solution compared to untreated skin. The placebo formulation resulted in negative or no reduction (-30% which was assumed as 0% reduction) in nicotine from green tobacco leaf extract. The two formulations (A+ and B+) were determined to be ideal for future studies to test protectiveness of gloves for use in potential clinical applications and field testing.

Formulation coated gardening gloves tested for protectiveness against green tobacco leaf extract

We further evaluated the use of coating Formulation B+ on gardening gloves since Formulation B+ showed the highest percent of reduction in nicotine permeation. Brown gloves containing Formulation B+, control brown gloves and white gloves with Formulation B+ showed a significant decrease in nicotine permeation (12.56 ± 3.62, 26.99 ± 4.53 and 26.07 ± 10.95 nmol, respectively) (Figure 3, Table 2) in the cumulative amount of nicotine at 24 h, compared to untreated skin (93.45 ± 27.24 nmol). Lag time for cumulative permeation of nicotine through untreated skin was 1.5 ± 0.8 h; lag times for brown gloves containing Formulation B+, control brown gloves, white gloves with Formulation B+ and control white gloves were 2.4 ± 1.6 h, 5.0 ± 1.5 h, 4.0 ± 2.3 h and 2.5 ± 1.7 h, respectively (Table 2). Lag times were not significantly

different compared to the untreated skin. The percent reduction for brown gloves containing Formulation B+, control brown gloves and white gloves with Formulation B+ and control white gloves were calculated to be 86.6%, 71.1%, 72.1% and 41.8%, respectively, compared to untreated skin. However, brown gloves coated with Formulation B+ did not show a significant difference compared to plain brown gloves, even though the cumulative amount and percent reduction of permeated nicotine over 24 h was lower in comparison. Control white gloves did not show a significant decrease in permeation (54.41 ± 20.28 nmol) compared to untreated skin, where the percent reduction of cumulative amount at 24 h was 41.8%.

Table 2 Barrier cream formulations tested against L-nicotine and green tobacco leaf extract

Nicotine from green tobacco leaf extract as donor solution

Applied formulations	Cumulative amount at 24 h (nmol) Mean ± SD	Lag time (h) Mean ± SD	Percent reduction of cumulative amount at 24 h
Brown gloves (w/B+) (n=8)	*12.56 ± 3.26	2.4 ± 1.6	86.6 %
Brown gloves (n=8)	*26.99 ± 4.53	5.0 ± 1.5	71.1 %
White gloves (w/B+) (n=9)	*26.07 ± 10.95	4.0 ± 2.3	72.1 %
White gloves (n=5)	54.41 ± 20.28	2.5 ± 1.7	41.8 %
Untreated skin (n=8)	73.24 ± 20.25	1.5 ± 0.8	--

*Designates significant difference (p<0.05) compared to respective untreated skin values

DISCUSSION

The absorption of dissolved nicotine from wet tobacco leaves or sap during the harvesting process results in GTS causing a series of symptoms that may require immediate emergency care and treatment [1,8]. Within the U.S. there have been several reported incidences of GTS in tobacco producing states like Kentucky, Florida, Tennessee and North Carolina. Despite the severity of the situation, very little has been achieved in terms of protecting farmers and farm workers against GTS and regulating its effects. The current measures using protective clothing have shown to be extremely cumbersome and uncomfortable when working in warm climates, which is normally when the leaves are harvested [1,2,6,7]. Chemical-resistant gloves and

waterproof rain jackets/gear are mostly made from rubber, which are not practical to work with in hot conditions. Therefore, an alternate protective method of utilizing a barrier cream is needed to improve working conditions in the field. Although the skin's stratum corneum serves as an effective barrier and a first line of defense against potentially noxious substances, nicotine has properties that attain toxic levels in the blood stream due to overexposure to tobacco leaves leading to GTS. Nicotine exists in the tobacco leaves as a lipophilic free base; it has a molecular weight of 162.2 g/mole and is readily skin penetrable [4,5]. The skin barrier function is related to the lipid and keratin content and micro-structure within the stratum corneum; if compromised, this can lead to impairment of the barrier function. Therefore, in addition to protective gear or even as a substitute, barrier creams may act as a second or third line of defense to prevent penetration of nicotine through the layers of the skin and finally into the vasculature. Barrier creams often work as skin protectants, which are intended for workers and employees for use prior to working with potentially harmful environmental chemicals [12,13]. The effectiveness of barrier cream protectant formulations create a physical diffusion barrier, and the acidic nature of the cream may influence a change in the chemical properties of the skin permeable toxic materials rendering them less harmful [12,13]. Barrier cream formulations mentioned in this study were prepared from naturally occurring organic acids. Nicotine, when combined with an organic acid, for example tartaric acid, citric acid or ascorbic acid, forms a salt highly water soluble and less penetrable through the skin [4,5]. Thus by changing the properties of nicotine base into a salt when it comes in contact with the applied barrier cream, the nicotine absorption is impeded at the skin surface.

In this study, four different tested formulations of a barrier cream prepared from naturally occurring organic acids have been shown effective in reducing the amount of permeated nicotine

through skin. The data indicates application of a barrier cream to the skin surface may significantly reduce the permeation of nicotine through skin when exposed to nicotine for 24 h. Of the tested barrier creams, Formulations A+ and B+ were the most effective of the creams, and were able to block 59.5% and 64.0% of the cumulative amount of nicotine absorption from green tobacco leaf extract when compared to the cumulative amount measured from untreated skin, respectively. These two formulations were also effective in reducing the absorption of L-nicotine by 86% to 98% when compared to permeation across untreated skin. In the field, tobacco farmers and farm workers may apply this cream before starting their work day and continue with their routine. The barrier cream treatment should give the exposed skin the protection it needs from excessive and repeated nicotine exposure which will be further evaluated in a field study. These two formulations (A+ and B+) may also be ideal for future clinical studies to test their protectiveness against occupational GTS. However, a few possible drawbacks for this sort of topical formulation would be handling duration of wet tobacco leaves, sweating due to high temperatures, frequent hand washing and changing clothes regularly between harvesting shifts [1,6], which may potentially remove any barrier cream from the skin along with nicotine salts. Therefore, these processes would require a second application before heading back into the fields. Also, in order to understand the total effectiveness in the field the cream may be required to be applied on all exposure areas of the workers. Such methods may have some potential difficulty in implementing it effectively in the field. Therefore, it may be useful to incorporate formulations into clothing or gloves so workers could replace gloves when done. This may further reduce exposure to nicotine. Out of the tested formulations, Formulation B+ was chosen as the best fit considering it had the best performance when comparing percent reduction in nicotine concentration. Two different types of gardening gloves (brown thick gardening gloves

and white thinner material gardening gloves as shown in Figure 2A and 2B) were selected and coated with Formulation B+. The effectiveness of these coated gloves was tested against green tobacco leaf extract. The coated gloves showed an 86.6% (brown glove) and 72.1% (white glove) reduction in permeation when compared to untreated skin (no gloves) suggesting the formulation is stable and performs well when incorporated with an existing commercial product. However, the true effectiveness of such applications will be known only in the actual field setting, where the formulations will be tested for usability, side effects, long term storage and formulation stability. Future studies should test the effectiveness of two formulations (A+ and B+) of the barrier cream on human skin and gloves (coated with these two formulations) in the tobacco harvesting work setting so as to evaluate the true usefulness of these creams with tobacco farmers and farm workers. An acceptable nicotine topical formulation effective in reducing skin exposure to nicotine may also reduce the occurrence of GTS which should be also evaluated in these tobacco farmers and farm workers.

CONCLUSIONS

GTS is a prevalent problem among the population of tobacco farmers and farm workers. Current preventative measures are not practical and there is a need to develop feasible solutions. Topical barrier cream formulations tested here have shown greater protective capacity and may considerably reduce nicotine exposure of tobacco farmers and farm workers during tobacco leaf harvesting work. The data presented here indicates that application of a barrier cream to skin significantly reduced nicotine permeation through skin when exposed to nicotine for 24 h. Of the tested barrier creams, Formulations A+ and B+ were the most effective creams, and were able to block 59.5% and 64.0% of the cumulative amount of nicotine absorption from green tobacco leaf extract when compared to the cumulative amount measured from untreated skin, respectively.

These two formulations were also effective in reducing the absorption of L-nicotine by 86% to 98% when compared to permeation across skin without the applied formulations. Gloves coated with Formulation B+ showed an 86.6% (brown glove) and 72.1% (white glove) reduction in permeation when compared to untreated skin suggesting the effectiveness of the formulation lasts even when incorporated with existing commercial personal protective equipment.

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COMPETING INTEREST

None.

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FIGURE LEGENDS

Figure 1 (A) Actual permeated amount of nicotine (nmol) versus time (h) for 0.7 mg/mL L-nicotine donor solution, (B) actual permeated amount of nicotine (nmol) versus time (h) for

from green tobacco leaf extract, (C) cumulative permeation (nmol) of nicotine from 0.7 mg/mL L-nicotine through Yucatan minipig skin of untreated and Formulations A, B, A+ and B+ treated skin and (D) cumulative permeation (nmol) of nicotine from green tobacco leaf extract through Yucatan minipig skin of untreated and Formulations A+, B+ and placebo treated skin.

Figure 2 (A) String knitted cotton work glove, medium size, medium weight, light brown color, 7 gauge (brown glove). Arrow shows the square section that was cut precisely to fit inside the flow-through diffusion cell and sat on top of the skin during diffusion. (B) Cotton inspector reversible/unhemmed glove, medium weight, white color, 7 gauge (white glove).

Figure 3 (A) Actual permeated amount of nicotine (nmol) versus time (h) for nicotine from green tobacco leaf extract, (B) Cumulative permeation (nmol) of nicotine from green tobacco leaf extract through Yucatan minipig skin. Skin samples inside the diffusion chambers were either protected with a square section from gloves either treated or untreated with formulation B+ where w/B+ signifies treated gloves.

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"Determinants of Nicotine Exposure in Tobacco Harvesting Workers: A Pilot Study"

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Nchekwubechukwu Okafor

Determinants of Nicotine Exposure in Tobacco Harvesting Workers: A Pilot Study

At the American Industrial Hygiene Conference & Exposition

June 2016

AIHA
Protecting Worker Health

Biological Monitoring Committee

BEST STUDENT POSTER

Presented to

Vedant Gohil

Tobacco Harvesting Work, Exposure to Nicotine, Vital Signs and Nicotine Poisoning

At the American Industrial Hygiene Conference & Exposition

June 2016

The American Industrial Hygiene Association Social Concerns Committee

recognizes the poster presentation…

"Determinants of Nicotine Exposure in Tobacco Harvesting Workers: A Pilot Study"

by

Nchekwubechukwu Okafor

In appreciation for a significant contribution to technical knowledge in areas of Social Concern in the field of Occupational Safety and Health

As presented at the 2016
American Industrial Hygiene Association Conference and Exposition
Baltimore, Maryland

Penney Stanch, MS, MPH, CIH, CSP
Chair, Social Concerns Committee

EQUIPMENT INVENTORY LIST AUTHORIZATION/PURCHASE

Report Date: 11/28/2016
Project Title: Efficacy Study of A Nicotine Barrier Cream
Grantee Name: UNT Health Science Center
Grants Management Officer: Ralph U Robinson

Grant Number: 5R03H009815-02
Project Period: 9/1/2014 to 8/31/2016
Project Officer: Maria Lioce
Grants Specialist: Bradis Belser

Description of Item (i.e., pH Meter)	Mfr.[1] (i.e., Fischer)	Serial Number	Quantity	Condition	Location	Purchase Cost	Date Received
Negative Equipment Report	Click here to enter text.	Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.		Click here to enter a date.
Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.		Click here to enter a date.
Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.	$Click here to enter text.	Click here to enter a date.
Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.		Click here to enter a date.

[1]Mfr. (Manufacturer)

Property Administrator & PO Disposition Recommendation and Instructions:

Description of Item (copy from above)	Disposition	Address[1]
	Choose an item. Click here to enter text.	Centers for Disease Control & Prevention Peachtree Distribution Center 3719 North Peachtree Road, #100
Click here to enter text.	Choose an item. Click here to enter text.	

Description of Item (copy from above)	Disposition	Address[1]
Click here to enter text.	Choose an item. Click here to enter text.	Chamblee, GA 30341
Click here to enter text.	Choose an item.	

1The CDC Warehouse is the central receiving point for the delivery of all non-hazardous and non-perishable supplies and equipment, CDC – AM – 2004-03, update 2010

Form Approved Through 10/31/2018
OMB No. 0925-0002

Department of Health and Human Services

Final Invention Statement and Certification

(For Grant or Award)

DHHS Grant or Award No.
5R03OH009815-02

A. We hereby certify that, to the best of our knowledge and belief, all inventions are listed below which were conceived and/or first actually reduced to practice during the course of work under the above-referenced DHHS grant or award for the period

09/01/2013 *original effective date* through 08/31/2016 *date of termination*.

B. Inventions (Note: If no inventions have been made under the grant or award, insert the word *"NONE"* under

NAME OF INVENTOR	TITLE OF INVENTION	DATE REPORTED TO DHHS
Youcheng Liu	NONE	
(Use continuation sheet if necessary)		

C. Signature — This block ***must*** be signed by an official authorized to sign on behalf of the institution.

Title
Assistant Vice President Research

Typed Name
LeAnn S. Forsberg

Signature
LeAnn S. Forsberg

Date
11/28/16

Name and Mailing Address of Institution
UNT Health Science Center
3500 Camp Bowie Blvd.
Fort Worth, TX 76107