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Nicotine Exposure and Decontamination on Tobacco Harvesters' Hands

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Green tobacco sickness is an illness associated with nicotine exposures among tobacco harvesters. Agricultural workers manually harvest tobacco and thus have the potential for skin exposure to nicotine, particularly on the hands. Often gloves are not worn as it hinders the harvesters' ability to harvest the tobacco leaves. The purposes of this study were to measure the concentration of nicotine residue on the hands of tobacco harvesters and the effectiveness of hand washing at removing the residue. Wipe samples from the hands of 12 tobacco harvesters were collected at the end of morning and afternoon work periods over two consecutive days. Each harvester had one hand wiped before washing his hands, and the other hand wiped after washing his hands with soap and water. Eight samples per worker were collected over the two days for a total of 96 samples collected. In addition to the hand-wipe samples, leaf-wipe samples were collected from 15 tobacco plants to estimate the amount of nicotine residue on the plants. The average nicotine level in leaf-wipe samples was 1.0 $\mu g\ cm^{-2}$. The geometric mean pre-wash and post-wash nicotine levels on the hands were 10 and 0.38 $\mu g\ cm^{-2}$, respectively. Nicotine leaf-wipe level, right or left hand and time of sampling did not significantly influence exposure. Job position—working on the bottom versus the top of the tobacco harvesting machine—was associated with nicotine levels. Pre-wash nicotine levels were higher for workers on the bottom of the harvester but not significantly higher ($P = 0.17$). Post-wash nicotine levels were significantly higher for workers on the bottom of the harvester ($P = 0.012$). A substantial amount of nicotine was transferred to the hands, but washing with soap and water in the field significantly reduced nicotine levels by an average of 96% ($P < 0.0001$).

Keywords: green tobacco sickness; hand exposure; hand washing; nicotine; tobacco harvester

INTRODUCTION

Green tobacco sickness (GTS) is characterized by nausea, vomiting, headache, and dizziness and is associated with nicotine exposures among tobacco harvesters (Gehlbach *et al*., 1975; Ballard *et al*., 1995; Arcury *et al*., 2001a, Trapé-Cardoso *et al*., 2003). Nicotine is a water and lipid soluble alkaloid found in tobacco leaves (Dawson and Solt, 1960) and harvesters who manually collect tobacco leaves absorb nicotine through the skin due to foliar contact.

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During the tobacco harvesting process, a worker's hands and forearms receive the most exposure (CDC, 1993). Increased levels of nicotine and cotinine have been measured in biological samples of exposed workers versus non-exposed workers (Gehlbach *et al*., 1975; D'Alessandro *et al*., 2001; Quandt *et al*., 2001). In particular, harvesting has been associated with the highest salivary cotinine levels among all tasks performed by tobacco farm workers (Quandt *et al*., 2001). Tobacco harvesting generally occurs between July and September and in 2003 ∼416 000 acres of tobacco were harvested in the US (USDA, 2004).

In general, tobacco harvesters have little control over most risk factors for nicotine exposure (Arcury

Fig. 1. Tobacco harvesting machine.

et al., 2001b). Traditional administrative and engineering controls are not feasible and personal protective equipment (PPE) is rarely worn. Wearing rubber gloves appears to reduce nicotine absorption (Ghosh *et al.*, 1991); however, impermeable gloves are not often used due to hot weather, loss of dexterity which hinders the ability to harvest and lack of availability. One possible exposure mitigation measure is to provide hand washing facilities for field workers. Wash stations in farm fields in the U.S. are mandated by Occupational Safety and Health Administration (OSHA) field sanitation regulations and the US Environmental Protection Agency (EPA) Worker Protection Standard (OSHA, 1987; EPA, 1992). Studies have shown that hand washing is effective at removing pesticides from skin (Campbell *et al.*, 2000; Curwin *et al.*, 2003). However, to date, no data are available showing whether hand washing in the field is effective at removing nicotine from the hands of tobacco harvesters.

The primary purpose of the study was to ascertain whether hand washing with soap and water in the field was an effective means of removing nicotine residue from the hands of tobacco harvesters. The objectives were 2-fold: (i) to estimate the extent of nicotine residue on the hands of harvesters and (ii) to estimate the amount of nicotine that was removed from the hands after washing with soap and water.

METHODS

Sample collection

Two crews of six tobacco harvesters each were enrolled in the study. The harvesters were Hispanic males harvesting the bottom three or four leaves of flue-cured tobacco in five fields near Kinston, North Carolina on 16 and 17 July 2001. Each crew rode on a tobacco harvesting machine, which moved slowly down the tobacco rows. Four harvesters seated near the ground collected leaves and placed them onto conveyor belts, and two workers on a platform above them took the leaves from the conveyor belts and placed them in a bin at the rear of the machine (Fig. 1).

Hand-wipe samples from each hand were obtained from 12 workers at four sampling times (day 1 morning, day 1 afternoon, day 2 morning and day 2 afternoon) for a total of 8 samples per worker and an overall total of 96 samples. Each sample was collected after ~4 h of work. For samples collected in the morning on day 1, workers were randomized to have both hands sampled either pre-wash or post-wash. This approach was initially chosen to account for potential handedness of the harvesters. However, upon observing the harvesters, it became apparent that right and left hands contacted the tobacco leaves equally. Therefore, for all the other sample periods, the sampling method was changed to account for inter-worker variability by randomizing the workers to have either their right hand sampled pre-wash then their left hand sampled post-wash, or their left hand sampled pre-wash then their right hand sampled post-wash. The workers were instructed to wash their hands as they normally would. Workers were observed washing their hands with liquid soap and a large container of water located on the tobacco harvester machine.

The hand-wiping method described in Geno *et al.* (1996) was used to sample for nicotine residue. This method had good recovery for several pesticides and has been employed in other studies investigating hand exposure to pesticides, including water soluble pesticides. The method is easy to implement in the field and it was felt that this method would be suitable for sampling nicotine residues on hands. The method involved wiping one hand with a 10 × 10 cm Sof-Wick® dressing sponge (Johnson & Johnson, Arlington, TX) moistened with 10 ml of 100% isopropanol, then wiping each finger of the same hand with a second dressing sponge. Investigators put on a clean pair of nitrile gloves before collecting wipe samples. Both sponges were placed in the same sample jar for analysis.

In addition to hand-wipe samples, tobacco leaf-wipe samples were collected from four of the five fields harvested. Three tobacco plants were sampled in three of the harvested fields, while six plants were sampled in the fourth field where both crews worked (total of 15 plants). A 200 cm^2 area on the top of selected tobacco leaves was wiped. Two leaves per plant, a bottom leaf and an upper leaf, were wiped and combined into one sample. To wipe a leaf, a 10 × 20 cm plastic template was placed on top of the leaf surface and held in place using four small clamps. A 10 × 10 cm Sof-Wick® dressing sponge, moistened with 10 ml of 100% isopropanol was folded in half and wiped over the length of the template three times. The sponge was then folded back onto itself, so that a 10 cm wide clean surface was presented, and a second wipe, perpendicular to the first was taken over the width of the template three times. The sponge was again folded so that a 10 cm wide clean surface was available and a third wipe, adjacent to the second and perpendicular to the first, was done three times. A clean pair of nitrile gloves was worn for each sample collected.

Sample extraction and analysis

Each sample, consisting of two gauze dressing sponges (wipes), was placed in a 22 ml accelerated solvent extractor (ASE) cell. The wipes were extracted on the ASE (Dionex, Inc.) at 2000 psi and 100°C using 1:1 hexane:acetone through two extraction cycles. The 25 ml extract was diluted to 40 ml and 4 ml (10%) was removed and stored at −15°C until analysis. The 4 ml aliquot was concentrated to 1 ml in a Kuderna-Danish evaporator and then solvent-exchanged into ethyl acetate and reconcentrated to a final 1 ml volume. Each extract was spiked with 100 ng of the internal standard dibromobiphenyl (10 μl of a 10 ng μl^{-1} solution), mixed, and analysed using gas chromatography with a mass spectrometer and multiple ion detection (GC/MS/MID). A multiple-point calibration curve was generated and these calibration solutions were interspersed among samples in the analysis sequence. The internal standard method of quantification was used, with a linear calibration curve generated using a least squares analysis of the calibration data.

For every 10 wipe samples extracted, one laboratory matrix blank (two wipes fortified with 1.5 ml isopropanol) was prepared in a manner identical to the sample preparation and these extracts were also analysed for nicotine. Once the range of nicotine in the field samples was ascertained from the GC/MS analyses, three spike levels were chosen for method recovery spike samples that represented approximately the low, middle and high ranges of analyte levels in field samples. These spike levels were 30, 300, and 3000 μg. Triplicate samples were prepared at each spike level.

Samples and standards were analysed using an HP 6890 GC/MS with a 30 m Rtx-35ms GC column (Restek), which had a 0.25 μm film thickness and a 0.25 mm internal diameter. The GC injection was made at 220°C; the column was initially held at 80°C for 2 min and then programmed at 15°C min^{-1} to 160°C, after which it was programmed at 5°C min^{-1} to 280°C. Total GC/MS run time per sample was ~31 min. The ions at *m/z* 133, 84, and 161 were monitored for nicotine. The retention time was ~10.3 min. The ions at *m/z* 312 and 314 were monitored for the internal standard, which had a retention time of ~22.5 min.

The quantity of nicotine in each extract was corrected for the 10% quantity of the initial extract that was carried through to analysis and for the average spike recovery of nicotine from the hand wipe media (55%). More specifically, the average percentage recovery from spiked samples were 59, 53, and 52 % at the 30, 300 and 3000 μg spike levels, respectively. The LOD was 0.1 μg per sample without correction for spike recovery and 0.2 μg per sample with correction for spike recovery. The recovery-corrected average level of nicotine in the laboratory matrix blanks was 0.3 ± 0.3 μg ($n = 12$) and ranged between not detected (ND) to 1.1 μg. The recovery-corrected average level of nicotine in the field blanks for the hand wipe samples was 0.5 ± 0.5 μg ($n = 10$) and ranged from ND to 1.6 μg. The recovery-corrected level of nicotine in the one field blank for the leaf-wipe samples was 0.2 μg.

Data analysis

All analyses used the recovery-corrected nicotine levels. Nicotine levels were converted from μg/sample to μg/cm^2 using a surface area of 420 cm^2/hand (EPA, 1997) for hand-wipe samples and 400 cm^2/leaf for leaf-wipe samples. A log (base 10) transformation was applied to the hand-wipe levels prior to statistical analysis. All analyses were performed using SAS version 8.2 (SAS Institute Inc., 1999). Descriptive statistics for leaf-wipe nicotine levels were computed by field. Analysis of variance (ANOVA) was used to test for differences among the fields. Descriptive statistics for pre- and post-wash hand-wipe nicotine levels were computed by sample, day and time. Since each worker had eight hand-wipe measurements taken over a 2 day period, the MIXED procedure in SAS was used to model hand-wipe nicotine levels treating worker as a random effect. Sample order (pre-wash, post-wash) was included in the models as a fixed effect to test for the effect of hand washing on hand-wipe nicotine levels. Additional fixed effects included sample time (day 1-morning, day 1-afternoon, day 2-morning, day 2-afternoon), sample hand (left, right) and harvester position on the tobacco harvesting machine (top, bottom). An interaction term between sample order and sample hand was used in order to determine if the effect of hand was different for pre- and post-wash samples. Similarly, interaction terms between sample order and sample time and sample order and harvester position were also used. Specifically, the model for the hand-wipe nicotine levels was given by:

$$\begin{aligned}\log(y_{ij}) = \beta_0 &+ \beta_1(\text{order}_{ij}\text{=post-wash}) \\ &+ \beta_2(\text{hand}_{ij}\text{=left}) \\ &+ \beta_3(\text{order}_{ij}\text{=post-wash})(\text{hand}_{ij}\text{=left}) \\ &+ \beta_4(\text{position}_{ij}\text{=below}) \\ &+ \beta_5(\text{order}_{ij}\text{=post-wash}) \\ &\times(\text{position}_{ij}\text{=below}) \\ &+ \beta_6(\text{time}_{ij}\text{=day1/morning}) \\ &+ \beta_7(\text{time}_{ij}\text{=day1/afternoon}) \\ &+ \beta_8(\text{time}_{ij}\text{=day2/morning}) \\ &+ \beta_9(\text{order}_{ij}\text{=post-wash}) \\ &\times(\text{time}_{ij}\text{=day1/morning}) \\ &+ \beta_{10}(\text{order}_{ij}\text{=post-wash}) \\ &\times(\text{time}_{ij}\text{=day1/afternoon}) \\ &+ \beta_{11}(\text{order}_{ij}\text{=post-wash}) \\ &\times(\text{time}_{ij}\text{=day2/morning}) + \gamma_i + \varepsilon_{ij}\end{aligned}$$

where y_{ij} is the hand-wipe nicotine level for subject i at time j; $i = 1, 2, \ldots, 12$; $j = 1, 2, 3, 4$; γ_i is the random effect for subject i and ε_{ij} is the random error term. Both compound symmetric and first-order autoregressive covariance structures were tested. Akaike's information criteria (AIC) were used to compare model fit. Results are presented as adjusted geometric means by taking the antilog of the adjusted log-transformed means. In addition, the relationship between hand- and leaf-wipe levels was assessed by adding a leaf-wipe mean term to the mixed-effects model of hand-wipe data. In this analysis, however, the sample size was reduced since leaf-wipe data was only available for four of the five fields where the harvesters worked. All significance testing was done at the 0.05 level.

RESULTS

Fifteen leaf-wipe samples were obtained from four of the five fields harvested. Nicotine levels in all 15 leaf-wipe samples were above the LOD. Nicotine levels on the leaves of the tobacco plants were not significantly different among the fields. Overall, the mean nicotine level for leaf-wipe samples was 1.0 μg cm^{-2}, with a range of 0.73–1.7 μg cm^{-2} (Table 1).

Ninety six hand-wipe samples were collected from 12 harvesters; however, eight samples from one harvester were excluded from analysis because the harvester wore gloves while using the harvesting machine. All of the remaining 88 hand-wipe samples were above the LOD (0.0004 μg cm^{-2}). Summary statistics for pre- and post-wash hand-wipe nicotine levels are presented in Table 2 by sample day and sample time and overall. The geometric mean (GM) nicotine level for the pre-wash hand-wipe samples was 10 μg cm^{-2}, with a range from 4.1 to 27 μg cm^{-2}. The GM nicotine level for the post-wash hand-wipe samples was 0.38 μg cm^{-2}, with a range from 0.02 to 2.2 μg cm^{-2}. On average, hand washing reduced the GM nicotine level by 96%.

Results of statistical modeling of the hand-wipe nicotine levels are presented in Table 3. The model utilizes a compound symmetric covariance structure, which provided a better fit than a first-order autoregressive covariance structure (not shown). While controlling for other factors, the results of the mixed model indicated that nicotine levels for post-wash samples were significantly lower than for pre-wash samples ($P < 0.0001$). Nicotine levels were similar on the right and left hands, both pre- and post-wash. Pre-wash nicotine levels were higher for workers on the bottom of the harvester, but not significantly higher ($P = 0.17$). Post-wash nicotine levels

Table 1. Summary statistics for measured leaf-wipe nicotine levels by field

Field	n	AM (μg cm^{-2})	SD (μg cm^{-2})	Range (μg cm^{-2})	Time harvested	Crew
1	6	1.17	0.30	0.86–1.73	Day 1—all day	1
					Day 1—afternoon	2
2	3	0.85	0.17	0.73–1.05	Day 2—all day	1
3	3	1.12	0.16	0.95–1.27	Day 2—morning	2
4	3	0.92	0.16	0.77–1.09	Day 2—afternoon	2
5	0	NA	NA	NA	Day 1—morning	2
Overall	15	1.05	0.25	0.73–1.73	—	

AM, arithmetic mean; SD, standard deviation; NA, not available.

Table 2. Summary statistics for measured pre-wash and post-wash hand wipe nicotine levels by sampling period

Day	Time	Sample order	n	GM (μg cm^{-2})	GSD	Range (μg cm^{-2})
1	Morning[a]	Pre-wash	12	9.90	1.41	6.62–19.39
		Post-wash	10	0.17	3.14	0.02–0.61
1	Afternoon[b]	Pre-wash	11	8.35	1.53	4.11–14.33
		Post-wash	11	0.74	1.90	0.29–2.21
2	Morning[b]	Pre-wash	11	11.64	1.36	5.97–18.44
		Post-wash	11	0.32	1.53	0.16–0.52
2	Afternoon[b]	Pre-wash	11	10.70	1.43	8.14–27.10
		Post-wash	11	0.46	1.64	0.22–1.04
	Overall	Pre-wash	45	10.07	1.45	4.11–27.10
		Post-wash	43	0.38	2.41	0.02–2.21

GM, geometric mean; GSD, geometric standard deviation.
[a]Workers were randomized to have both hands sampled pre- or post-wash.
[b]Workers were randomized to have either (i) right hand sampled pre-wash and then left hand sampled post-wash or (ii) left hand sampled pre-wash and then right hand sampled post-wash.

Table 3. Adjusted geometric mean nicotine levels (μg cm^{-2}) obtained from the mixed-effects model for hand-wipe samples[a]

Effect	Value	Pre-wash	Post-wash	P-value (pre versus post)
Hand	Right	9.1	0.37	<0.0001
	Left	9.1	0.31	<0.0001
	P-value (right versus left)	0.98	0.22	
Job	Top of harvester	7.8	0.25	<0.0001
	Bottom of harvester	11	0.45	<0.0001
	P-value (top versus bottom)	0.17	0.012	
Sampling time	Day 1 morning	8.3	0.18	<0.0001
	Day 1 afternoon	7.8	0.64	<0.0001
	Day 2 morning	11	0.29	<0.0001
	Day 2 afternoon	9.9	0.39	<0.0001

[a]The model uses $n = 88$ hand wipe samples from 11 workers. Eight samples from one harvester were excluded because the subject wore gloves while working on the harvester machine.

were significantly higher for workers on the bottom of the harvester ($P = 0.012$). Time of sampling did not significantly affect pre-wash nicotine levels, but post-wash samples obtained after the morning shift on day 1 were significantly lower than the other sampling times and post-wash samples obtained after the afternoon shift on day 1 were significantly higher than the other sampling times. Pre-wash hand-wipe levels did not show a linear trend over the four sampling times. Post-wash hand-wipe levels also did not exhibit a linear trend over time. Hand-wipe nicotine levels were not significantly related to field leaf-wipe means.

DISCUSSION

Nicotine was transferred to the hands of tobacco harvesters from repeated contact with tobacco leaves. Washing hands had a large and significant effect on the amount of nicotine residues remaining on the hands. Washing with soap and water reduced the residues on average 96%; therefore, using hand washing facilities can substantially reduce nicotine residues on hands.

The sample size was small, but no other data existed on the effectiveness of hand washing in the field at removing nicotine residue from the hands. Other studies have focused on pesticides and found that washing removed from 23 to 96% of pesticide residues, depending on the pesticide, the solvent used for cleansing, the time since exposure and the amount of pesticide originally on the skin (Pelletier *et al*., 1990; Brouwer *et al*., 2000; Campbell *et al*., 2000; Marquart *et al*., 2002; Curwin *et al*., 2003). Among the same workers investigated in this study, 96% of acephate residue, a water soluble organophosphate pesticide, was removed from their hands (Curwin *et al*., 2003).

The effect of hand washing on absorbed nicotine dose is not known. Biological monitoring for nicotine exposure would have been useful to determine the effect on nicotine absorption. Ultimately, protective measures to prevent green tobacco sickness should be aimed at reducing dermal absorption of nicotine and therefore nicotine dose. The hands are only one area on the body that may be exposed to nicotine and may not be the area most exposed. Harvesters can be exposed over large portions of their body despite wearing work clothing due to substantial contact with tobacco that may be wet with dew. Contact with wet tobacco and wearing work clothes that have become wet from dew or perspiration may increase exposure and absorption of nicotine through the skin. Additionally, dermal absorption tends to be less in the hands than other areas of the body. Dermal exposure sites will vary depending on the tobacco harvesting method used which varies with tobacco type. In particular, the axilla may receive a large amount of nicotine exposure during most manual harvesting whereby harvesters carry the tobacco leaves under their arm while harvesting. The harvesting method used in this study is often not employed during tobacco harvesting.

In an intervention study among tobacco harvesters in India, Ghosh *et al.* (1991) found that nicotine and cotinine in urine were reduced significantly when wearing gloves during harvesting. Further, nicotine and cotinine urine concentrations in harvesters not wearing gloves were significantly greater than an unexposed control group but were not significantly different when wearing gloves. However, gloves are infrequently worn during harvesting and only one worker of 12 wore gloves in this study. This worker had pre-wash hand wipe nicotine levels of 0.45, 0.56 and 1.52 $\mu g\ cm^{-2}$, which was substantially lower then the overall geometric mean pre-wash level of 10 $\mu g\ cm^{-2}$, while the post-wash level was similar.

Presumably, hand washing would have an effect on nicotine absorption, but to what extent is unknown. Zorin *et al.* (1999) found that nicotine permeation through skin *in vitro* continued despite removing almost all nicotine from the skin after 3 or 5 min. The cumulative permeation was lowest when the skin was rinsed after 3 min, indicating that early washing may be necessary. The flux and mean lag time for nicotine permeation through human skin ranged from 3 to 1342 $\mu g\ cm^{-2}\ hr^{-1}$ and 8–24 min, respectively, depending on the nicotine concentration, carrier vehicle and rinsing (Zorin *et al.*, 1999). The fact that the tobacco harvesters washed their hands shortly after they finished harvesting, coupled with the high water solubility of nicotine, might account for the high amount of nicotine removed. A delay in washing would result in more nicotine being absorbed into or bound to the skin, resulting in less nicotine being removed by hand washing and more of it being bio-available.

Harvesters' nicotine exposures varied depending on the job they performed. Harvesters on the bottom of the harvesting machine collecting the tobacco leaves tended to have greater hand exposure. They were in greater contact with the tobacco leaves and contacted tobacco leaves more frequently than the harvesters on the top of the machine. Others have shown that GTS and cotinine levels are related to the task performed by tobacco harvesters (Arcury *et al.*, 2001a; Quandt *et al.*, 2001).

The laboratory recovery of nicotine spiked on the wipe media averaged 55% and is a limitation of this study. However, laboratory recovery was similar across different nicotine loadings and the values reported were corrected for the incomplete recovery. Therefore the limitation should not affect the overall conclusion. A more important limitation is that the nicotine removal efficiency of the hand and leaf-wipe method employed in this study was not determined. The exposure values could be underestimated due to incomplete removal, and hand washing effectiveness results could be affected if the wipe method removal efficiency is different for different nicotine loadings. If loadings such as those similar to the post-wash hand nicotine levels are not removed as efficiently as higher levels similar to the pre-wash levels, the effectiveness of hand washing demonstrated in this study may not be as great. However, if we assume that 100% of the nicotine was removed for the pre-wash samples and only 10% was removed for the post-wash samples, hand washing would have removed 65% of the nicotine residues.

It is unlikely that the difference, if any, in removal efficiency would have been that great. Geno *et al.* (1996) reported good removal efficiency for chlorpyrifos (104%) and pyrethrin (92%) using this wipe method on hands. Fenske *et al.* (1999) reported that hand wiping underestimated hand exposure for apple thinners exposed to azinphos-methyl. Brouwer *et al.* (2000) conducted a literature review on hand wipe removal efficiencies and reported a range of 36–104%.

If the removal efficiencies are the same at both pre-wash and post-wash levels, but are poor, the estimated exposure would be underestimated; however, there would not be a bias toward either the pre- or post-wash results. Since the effectiveness of hand washing at reducing exposure was the main goal of this study, underestimating exposure, provided there was no bias for either the pre- or post-wash results, would not impact on this goal.

CONCLUSION

Substantial amounts of nicotine residue were transferred to the hands from tobacco leaves during harvesting. Hand washing with soap and water removed a significant amount of nicotine residue from the hands, but the effect on nicotine absorption is not known. Nicotine removal efficiency for the wipe method was not determined and is a limitation of this study. Future research should focus on determining the ability of hand washing to reduce nicotine absorption through the skin and the frequency of hand washing necessary to be effective.

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