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Environmental and Behavioral Predictors of Salivary Cotinine in Latino Tobacco Workers

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We report the results of a cohort study of 182 seasonal and migrant **farmworkers** engaged in tobacco production in two North Carolina counties. Data were collected on tobacco work tasks and risk factors for exposure to nicotine, including smoking, every 2 weeks over a 10-week period during the summer of 1999. Saliva samples were collected for cotinine analysis at every contact. Salivary cotinine levels

increased across the season, independent of smoking status. Multivariate analyses identified a model ($R^2 = 0.68$) in which predictors of cotinine included greater age, later-season work, wet working conditions, smoking, and work task. Harvesting ("priming") tobacco was associated with higher cotinine levels than other tasks. This study demonstrates that tobacco workers experience substantial work-related exposure to nicotine. The long-term effects of such exposure should be investigated.

Introduction

Research from tobacco-producing regions throughout the world reports that workers participating in hand labor in tobacco fields experience a syndrome called green tobacco sickness (GTS). [1] [2] GTS is characterized by nausea, vomiting, headache, and dizziness in workers exposed to tobacco. [3] [4] [5] Although GTS is normally self-limiting, it can result in dehydration requiring hospitalization, [6] and seizures have been reported. [7] As tobacco cultivation shifts from small plots worked by family members to large holdings worked by migrant and seasonal **farmworkers** or to overseas production, [1] the burden of GTS shifts. Factors reported in the literature to increase the likelihood of GTS include working in wet tobacco, performing work tasks that result in body contact with the plants, and younger age. [3] [4] [8] Protective factors reported include the use of protective clothing and tobacco use (smoking and smokeless). [3] [4] [8] The cause of this occupational illness has been attributed to nicotine poisoning resulting from transdermal absorption of nicotine from the leaves of the mature tobacco plant. [5] However, there have been no studies to verify the absorption of nicotine resulting from tobacco work, to document levels of absorption, or to explore risk factors for such absorption.

This investigation reports the results of a cohort study of Latino migrant and seasonal **farmworkers** engaged in flue-cured tobacco production over the course of one summer in North Carolina. Salivary cotinine was used as a measure of nicotine exposure. The objectives were to (1) describe the salivary cotinine levels of workers across the season; and (2) identify behavioral and environmental factors that regulate the level of salivary cotinine in workers, with particular attention to the combination of the degree of smoking and the type of tobacco work performed.

Materials and Methods

Population and Study Design

This study used a longitudinal surveillance design to collect information on salivary cotinine and GTS risk factors from a cohort of **farmworkers** at biweekly intervals over a 10-week period. The design and population are described in detail elsewhere. [8] Briefly, data were collected in Wake and Granville Counties, North Carolina; North Carolina is the largest tobacco producer in the United States. [9] Both Wake and Granville Counties are in the flue-cured tobacco region and were ranked 4th and 15th, respectively, of the state's 100 counties in total tobacco production in 1999. [10]

The study was designed to include the random selection of 18 **farmworker** residence sites from each of the two counties. These 36 sites were randomly selected from lists compiled by the migrant clinics in each county from previous years' experience. All sites were visited, a census was taken, and preliminary consent was obtained from residents. Twenty-nine of the originally selected sites were not in use and were randomly replaced. At no inhabited site did residents refuse to participate. Because no African American workers and very few women were found to be living at the sites, random recruitment proceeded without regard for ethnicity and gender. The original plan called for randomly recruiting five **farmworkers** per site to produce a sample of 180. The recruitment plan was modified because some sites had fewer than five residents, some experienced considerable turnover in workers, and one site was abandoned early in the study. At smaller sites, all **farmworkers** were recruited. For sites with five or more residents: at eight sites up to seven workers were recruited, at two sites four participants were recruited, and at 13 sites five participants were recruited. At the end of the initial selection and recruitment period, the sample included 168 **farmworkers** from 36 sites.

Between the initial period and the first follow-up, one site was abandoned by its residents, and a 37th site was selected for participation. For the third through final follow-up interview periods at sites with worker turnover, new residents were randomly selected and recruited if more than three sampled residents from that site were reported by other residents to have permanently left the site. If a sampled resident who had not permanently left the site could not be located for an interview within 2 days before and 3 days after the scheduled 2-week follow-up, this interview was not completed.

The total sample included 187 **farmworkers**. Of these, five were excluded from analysis because they had never worked in tobacco during the entire data collection period. This left a final sample of 182 **farmworkers**, with 701 data points. The resulting sample included 98 **farmworkers** from whom data were collected at each

of five interviews; 16 **farmworkers** who were recruited at the first period but with whom no follow-up interviews were completed; 50 **farmworkers** who were recruited at the first period and with whom between one and three follow-up interviews were completed; and 18 **farmworkers** who were recruited after the first period and with whom between one and three follow-up interviews were completed. This sample included 178 Hispanic men, three Hispanic women, and one white non-Hispanic man.

Data Collection

Interviewing began on June 21, 1999, and was completed on September 5, 1999. The data collection procedures were reviewed and approved by the Institutional Review Boards of Wake Forest University School of Medicine, University of North Carolina at Chapel Hill, and the Centers for Disease Control and Prevention. During the first interview period, sites were visited and specific **farmworkers** were selected and asked to participate. Signed informed consent was obtained.

The interviews were conducted after work hours in the language of choice of the participant, with English and Spanish questionnaires available for use. Baseline interviews took about 20 minutes to complete. At approximately 2-week intervals, the interviewers returned to each site and conducted the follow-up interviews, which took approximately 15 minutes to complete.

The interview questionnaires (baseline and follow-up) were developed in English to ensure that all topics important to the data collection were included. The questionnaires and the consent form were translated into Spanish by a professional translator familiar with Mexican Spanish and with **farmworkers** in North Carolina. The questionnaires and consent form were then reviewed by native Spanish speakers from Mexico who had been **farmworkers** and were pre-tested with eight **farmworkers**. During the pre-test, **farmworkers** were first asked to answer an item and then to comment on what the item meant. Based on the pre-test results, the investigators and the field interviewers reviewed each questionnaire item and made final revisions.

At each interview, each participant provided a saliva sample by chewing a sterile swab insert from a Salivette (Sarstedt, Newton, NC) until it was thoroughly soaked. The swab was replaced in the plastic tube without handling and was labeled. The tubes were immediately placed in a cooler with ice packs for transport to laboratory freezers at -20°C. Samples were collected and shipped biweekly on dry ice to

Centers for Disease Control laboratories in Atlanta. These samples were subsequently thawed and centrifuged. The samples were extracted and the level of cotinine was measured using a sensitive atmospheric-pressure ionization, tandem mass spectrometric (LC-API-MS-MS) method developed previously, [11] applied to The Third National Health and Nutrition Examination Survey [12] and described elsewhere. [13] Cotinine was measured for 694 of the 701 data collection points. In three cases, the swab contained a quantity of saliva insufficient for analysis. In the four remaining cases, mass spectral data suggested the presence of a potential interference in the analysis.

Questionnaire Content

Cotinine predictors can be divided into two sets: those characteristics expected to be unchanged across the study period and collected only in the baseline interview, and those characteristics and behaviors that might change across the study period and were collected at baseline and each follow-up interview. Baseline characteristics included age, BMI (body mass index), educational attainment, ability to understand English, years of tobacco work experience, self-rated health, and immigration status (under a work contract, or H2A visa). Gender and ethnicity were collected but were not considered in this analysis because there was no variability in these characteristics. The follow-up characteristics included type of work with tobacco, skin integrity, recent tobacco use, living with a smoker, working in wet clothing, working without a shirt, wearing a rain-suit, taking measures to prevent GTS, and changing out of wet clothes.

The baseline characteristic BMI was calculated as weight in kilograms ÷ (height in meters)^2 from height and weight measurements collected at the initial interview to which a standard formula was applied. "Understand English" was based on a question that asked respondents to rate their ability to understand English with the categories none, very little, some, most, and all. Only four workers stated that they understood all or most English. Therefore, responses for this measure were collapsed into two categories: understood some English and understood no English. For self-rated health, respondents were asked to rate their health with the categories excellent, very good, good, fair and poor. These categories were collapsed for the analysis into the two categories, "very good, excellent" versus "poor, fair, good."

At each interview, respondents were asked the average number of cigarettes smoked, cigars smoked, or pinches of snuff or chewing tobacco taken per day over the previous week. Most workers reported tobacco use as cigarette smoking. Compared

with a total of 1278 cigarettes reported, there were only 18 cigars and 25 instances of chewing tobacco use reported. Therefore, each cigar smoked and tobacco chew was considered as equivalent to a cigarette smoked. Interest is focused on the number of cigarettes smoked in a week. For descriptive purposes, we also examined a tobacco use status variable in which each of the 694 observations in the analysis data set were classified into one of four categories according to the dichotomous variable of any cigarettes smoked for the week in question and in relation to the smoking status of the other weeks recorded. AS refers to "always a smoker" and includes all observations from the group of workers who reported tobacco use of any kind at each of the survey occasions for which they were interviewed. NVS refers to "never a smoker" and includes all observations from the group of workers who never reported having used tobacco during the weeks in the summer of 1999 that corresponded to their survey occasions. Finally, observations from the group of occasional smokers, or those who reported tobacco use on at least one but not all survey occasions, were broken down into two categories. AOS ("active occasional smoker") includes observations from occasional smokers who reported having used tobacco during the week queried by the given survey, whereas IOS ("inactive occasional smoker") consists of observations from occasional smokers who reported in the week before the survey that they were non-smokers and non-tobacco users.

Several variables in this study varied daily. A key variable was the primary type of tobacco work performed. For each of the 7 days queried, the **farmworker** reported the number of hours spent working in tobacco and whether the respective tasks of topping, priming, and barning were performed. Topping refers to breaking the flower off the top of the plant. Priming refers to actually picking or harvesting the tobacco leaves. Barning refers to putting the harvested tobacco into a barn for curing. The number of hours spent on each task was not reported. A categorical variable with six levels referred to as type of work was determined as the task performed more often than any other during the 2-day period, including the day of and the day before the saliva sample. If only one task was reportedly performed on a given day, then the total hours worked for that day were attributed to the task; otherwise, the total hours worked in a day were equally apportioned among the two or three tasks indicated. A category of Priming/Barning was created because, often, these two tasks were performed on each of 2 consecutive days and the actual primary activity, if there was one, could not be determined. When neither topping, priming, nor barning were reported, type of work was defined as "Did not work" if zero hours of tobacco work were reported and "Other" if more than zero hours were reported. "Other" refers to any other activity, such as driving a tractor. Also, when hours worked exceeded zero but work indicators for the primary three activities were missing, we treated this as Other.

Wet was defined as yes if the work conditions of either the day of or the day before the interview were reported as wet. Hours worked were the total number of work hours reported for those same 2 days. Hours worked in wet clothes were defined similarly. For those 330 day-pairs in which workers reported working in wet clothes, we reported the percentage who said they change out of wet clothes (with one value missing). Work with no shirt refers to whether the worker worked without a shirt on either of the two days.

Skin integrity had the value "poor" if a worker had a scrape, a rash, or two or more cuts anywhere on the arms, axilla, or upper torso; the value "good" was applied if a worker had no scrapes or rash and no more than one cut. Wears a rain-suit indicated whether a worker responded affirmatively concerning wearing a rain-suit to prevent getting sick while working in tobacco. Finally, takes preventive measures indicated if a worker responded yes to taking medicine or doing anything else to prevent getting sick while working with tobacco.

The agricultural season was divided into three parts: early (June 21 to July 18), middle (July 19 to August 8), and late (August 9 to September 5).

Data Analysis

The descriptive analyses focus on the comparison of subgroup mean cotinine levels. We report weighted means computed for which each cotinine measurement has an associated weight based on the sampling design. The weight for a cotinine measurement obtained at a particular period for a given **farmworker** is the inverse of the product of that worker's probability of selection into the sample and probability of being a responder at the period. The probability of selection into the sample is a value that is constant across the observations of a worker and is calculated as the product of the probability of the selected farmworker's site being selected and the probability the worker was selected, given that his or her site was selected. The probability of being a responder is the fraction of workers sampled who worked in a period. This non-response adjustment is made by pooling observations of workers from similar sites according to geographic region.

All statistical tests reported in this article are based on survey linear regression techniques that account for the repeated measures on workers and clustering of workers within camps through their use of design-based variance estimators. [13] In the first step of our analysis, comparisons of subgroup means are based on the Wald statistic from a linear model for the natural log transform of cotinine, adjusting for

tobacco use status by its inclusion in the model. The natural log transform is used in the linear regression analyses because the distribution of cotinine is highly skewed. [14] Tobacco use status subgroups are compared in a similar fashion without adjustment for any other variable. In the survey linear regression analysis, the work camp is considered the primary sampling unit, because not only might we expect a worker's repeated measures to be correlated with one another, but we might also expect some intra-site correlation among the cotinine values of workers in the same camp.

In the second step of the analysis, those risk factors found to be statistically significant from simple two-variable linear models are considered as candidate explanatory variables for a multivariate linear model. A multistep procedure is used to identify a good-fitting final linear survey regression model that describes the variation in the logarithm of cotinine. First, a backward elimination procedure is used, starting with a model that has the main effects corresponding to the candidate variables. Once a good-fitting main effects model is identified, the second step tests the significance of adding each possible two-way interaction to this initial model using forward selection. Special attention is given to the relationship of the number of cigarettes smoked and cotinine level and how this relationship may depend on the type of tobacco work performed. Throughout, statistical significance is defined with P value less than 0.05.

Results

The sample consisted of 178 Hispanic men, three Hispanic women, and one non-Hispanic white man. All but the non-Hispanic man were born in Mexico and spoke Spanish. Fifteen reported that they spoke an indigenous, non-Spanish language at home. The participants ranged in age from 18 to 64 years (median, 27.6 years). The number of years working in tobacco ranged from 1 (the present year) to 25, with a mean of 4.6 years. Over half of the participants had come to the United States on a work contract.

Tobacco use (cigarettes, cigars, or smokeless tobacco) averaged 1.88 per day across the entire sample. The population estimate is the weighted sample mean of 2.19 (standard error, 0.42). Tobacco use greater than zero was reported for 261 of the 694 weeks. Per day use for those weeks ranged from 1 to 25, with the distribution

skewed toward fewer cigarettes. The median was four per day and the 75th percentile was six per day.

Table 1 reports weighted sample means, which are estimates of population mean cotinine levels for subgroups defined by smoking status. These means verify the smoking status classifications. Overall, for the 694 data points, the mean cotinine level was 76 (standard error, 7). Data points for the AS group averaged cotinine levels of 145 ng/mL, about twice as high as the mean cotinine levels for occasional smokers on data points for which smoking was reported (AOS group). The mean cotinine level was higher for the AOS data points than for those of occasional users who were current non-users (IOS group). Finally, this last group of data points had higher mean cotinine levels than data points for those who never used tobacco during the weeks they were surveyed (NVS group). The overall *P* value for any subgroup differences was less than 0.001, and all six pairwise comparisons had a probability level of < 0.05.

Table 1. Cotinine Means (in ng/mL) and SE and Baseline Characteristics [a]

Value	No. of Surveys	No. of Workers		Overall (*n* = 694) (mean/SE)	AS (*n* = 153)	Smoking Status (mean/SE)		
		n	%			AOS (*n* = 114)	IOS (*n* = 113)	NVS (*n* = 314)
Overall [**]	694	182	100.0	76 /7	145 /20	75 /11	57 /9	46 /7
BMI								
<25	241	66	36.3	84 /11	153 /37	77 /15	63 /16	53 /13
25 to <30	331	87	47.8	67 /7	116 /11	65 /24	55 /10	45 /9
≥30	122	29	15.9	87 /25	234 /34	84 /17	44 /14	41 /12
Age [*]								
18-24	149	46	25.2	48 /7	70 /15	47 /13	42 /9	43 /9
25-34	305	78	48.9	88 /10	165 /29	79 /15	76 /18	51 /9

Value	No. of Surveys	No. of Workers			Smoking Status (mean/SE)			
		n	%		AS (*n* = 153)	AOS (*n* = 114)	IOS (*n* = 113)	NVS (*n* = 314)
≥35	240	58	31.9		153 /28	96 /24	43 /10	44 /8
Education								
0-5 yr	245	58	32.0	85 /12	184 /30	62 /10		43 /9
6-8 yr	229	64	35.4	68 /11	112 /20	72 /25		49 /10
9-16 yr	215	59	32.6	76 /11	131 /46	97 /18		47 /11
Understand English								
Some	336	93	51.1	79 /9	128 /19	90 /18		42 /7
None	358	89	48.9	74 /10	176 /33	62 /14		49 /9
Years worked in tobacco								
First	198	59	32.4	79 /12	126 /27	65 /26	53 /14	50 /11
2-4	291	75	41.2	67 /10	97 /23	79 /12		51 /10
≥5	205	48	26.4	87 /16	199 /29	76 /24		37 /9
Self-rated health								
Very good, excellent	333	86	47.3	81 /7	147 /23	92 /17		50 /8

Value	No. of Surveys	No. of Workers		Smoking Status (mean/SE)				
		n	%	Overall (*n* = 694) (mean/SE)	AS (*n* = 153)	AOS (*n* = 114)	IOS (*n* = 113)	NVS (*n* = 314)
Poor, fair, good	361	96	52.7	72 /10	144 /29	61 /13	43 /7	42 /10
Work contract*								
Yes	377	99	55.3	82 /12	159 /30	59 /12	60 /9	56 /9
No	306	80	44.7	69 /6	124 /21	84 /17	55 /13	34 /9

[a] SE, standard error; AS, always smoker; AOS, current and occasional smoker; IOS, non-current but occasional smoker; NVS, never smoker; BMI, body mass index. Subgroup comparisons for means in the "Overall" column adjusting for smoking status;

* $0.001 < P < 0.05$.

** $P < 0.001$

There were no significant subgroup differences according to BMI, education, whether or not a worker understands English, years worked in tobacco, or self-rated health, after adjusting for variations according to smoking status (Table 1). Age greater than 24 years was significantly positively associated with a higher mean cotinine level ($P = 0.013$). **Farmworkers** with a work contract had significantly greater mean cotinine levels than those without ($P = 0.033$).

There was a highly significant increase in mean cotinine levels as the season progressed (Table 2, Fig. 1). Furthermore, this progression was evident in data points for every smoking status group. Table 2 reports that skin integrity, living with a smoker, and working with no shirt were not significantly associated with cotinine after adjusting for variation in cotinine level due to smoking status. However, for the

NVS group, the average cotinine level was twice as high (3.51 ng/mL vs 1.76 ng/mL) for those who lived with smokers versus those who did not live with smokers for periods of no work exposure ($n = 31$ and $n = 22$, respectively). Hours worked was significantly related to cotinine, with the apparent major difference between those who did not work at all (0 hours worked) and those who worked. Working in wet conditions was significantly positively related to cotinine levels ($P < 0.001$). The number of hours working in wet clothes was significantly positively associated with cotinine ($P < 0.001$). Those who changed out of wet clothes had significantly higher cotinine levels than those who did not ($P < 0.001$), although there was little to no difference for the NVS group. Those who reported wearing a rain-suit or adopting other preventive measures (including taking medicines) had higher average cotinine levels overall. However, these results were not consistent across tobacco use groups. Generally, the mean cotinine values shown in table 1, table 2 suggest that interactions with smoking may be present. These interactions are assessed with the continuous variable of the number of cigarettes smoked in a multivariable linear regression.

Table 2. Cotinine Means (ng/mL) and SE and Changeable Characteristics of Workers [a]

Value	No. of Surveys	Overall (n = 694) (mean/SE)	Smoking Status (mean/SE)			
			AS (n = 153)	AOS (n = 114)	IOS (n = 113)	NVS (n = 314)
Overall [**]	694	76 /7	145 /20	75 /11	57 /9	46 /7
Type of work [**]						
Prime	90	150 /18	203 /34	95 /24	102 /16	141 /23
Prime/barn	127	119 /10	165 /22	126 /27	102 /21	103 /19
Barn	69	69 /13	120 /26	88 /33	25 /8	33 /7
Top	210	51 /8	152 /37	51 /12	19 /7	11 /1
Other	97	46 /9	127 /19	84 /25	18 /7	17 /9
Did not work	101	28 /7	104 /29	35 /7	3 /2	3 /1
Skin integrity						

Value	No. of Surveys	Overall (n = 694) (mean/SE)	Smoking Status (mean/SE)			
			AS (n = 153)	AOS (n = 114)	IOS (n = 113)	NVS (n = 314)
Good	591	58 /11	154 /21	77 /12	58 /10	45 /7
Poor	103	79 /7	79 /26	45 /17	52 /13	54 /16
Hours worked**						
0	101	28 /7	104 /29	35 /7	3 /2	3 /1
≤8	120	72 /15	155 /33	110 /24	35 /11	43 /15
≤16	257	92 /12	169 /26	69 /12	71 /17	53 /11
>16	216	80 /9	118 /29	86 /19	64 /15	63 /18
Live with smoker						
Yes	480	81 /10	145 /21	79 /13	55 /12	45 /10
No	211	67 /9	148 /36	70 /19	59 /15	49 /8
Wet**						
Yes	385	87 /9	145 /24	88 /17	70 /12	64 /9
No	309	64 /8	146 /24	59 /10	42 /11	26 /8
Hours work in wet clothes**						
0	364	65 /8	147 /26	61 /9	39 /10	28 /7
≤5	174	67 /11	118 /27	96 /25	35 /10	47 /10
>5	156	112 /13	166 /29	89 /22	111 /20	92 /16
Change wet clothes**						
Yes	223	100 /12	175 /29	101 /20	87 /17	68 /11
No	106	65 /11	75 /17	71 /24	42 /13	65 /19
Work with no shirt						
Yes	23	71 /18	50 /7	165 /47	66 /41	59 /19

Value	No. of Surveys	Overall (*n* = 694) (mean/SE)	Smoking Status (mean/SE) AS (*n* 153)	AOS (*n* 114)	IOS (*n* = 113)	NVS (*n* 314)
No	671	77 /7	149 /21	73 /10	57 /9	46 /7
Wear rain-suit*						
Yes	207	88 /10	142 /21	/21	76 /14	
No	18	77 /7	147 /23	64 /11	50 /10	
Preventive measures*						
Yes	51	93 /16	101 /4	129 /31	49 /22	95 /24
No	643	75 /7	149 /21	73 /11	57 /9	4 /7
Period**						
Early	256	35 /6	104 /21	50 /8	10 /4	
Mid	213	70 /10	14 /28	4 /12		
	225	18 /10	179 /20	119 /21	96 /17	9

[a] For definition of abbreviations, see Table 1. Subgroup comparisons of means in the "Overall" column adjusting for smoking status;

* $0.001 < P < 0.05$,

** $P < 0.001$

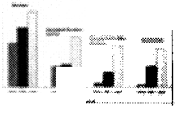

Fig. 1. Mean salivary cotinine levels (ng/mL), by work period and smoking status, for **farmworkers** in North Carolina, 1999.

The final multivariate model shown in Table 3 had $R^2 = 0.68$. Older age, having a work contract, and working in wet conditions were significantly related to higher

levels of cotinine. Mean cotinine levels increased as the tobacco season progressed from early, to middle, and then to late season. The model also included the main effects of type of work, separate linear effects of smoking for each type of work (smoking by work type interaction), and a quadratic effect of smoking for one of the work type subgroups, the group that did not work. Quadratic effects for the remaining work type subgroups were not statistically significant. Specifically, we modeled the linear effect of smoking as ln(1 + number of cigarettes) because the untransformed value had a skewed distribution. Because the model contains smoking by type of work interactions, the parameter estimates given in Table 3 for the type of work correspond to the effect of the primary type of work relative to the "did not work" reference group for current non-smokers, or 0 cigarettes smoked. As in Table 2, priming, followed by priming/barning, is significantly associated with higher cotinine levels in comparison with not working. The tasks of barning, topping, and "other" fall into a middle group, with expected cotinine levels higher than for those that did not work but less than that of workers involved in priming. The finding that "other" work results in cotinine levels similar in magnitude to levels for topping and barning is probably attributable to the occasional failure to accurately report priming, barning, or topping activities and our decision to treat missing values for these variables as "other" work when reported hours worked exceeded zero. As expected, a positive and statistically significant relationship was found between smoking and cotinine level for the group that does not work, given by the linear effect of 3.474 and the quadratic effect of -0.675. The relationship between smoking and cotinine in the context of different types of tobacco work is illustrated in Fig. 2 for the late season. Values on the fitted regression lines were considered unreliable for more than six cigarettes smoked per day because less than 5% of the 694 observations exceeded that number. Figure 2 shows fitted values of log cotinine for a worker 30 years of age, with a work contract, and working in dry conditions. For other subgroups defined by these variables, the relationship of the curves would stay the same but would be shifted up or down according to the subgroup's regression coefficients. For example, the graph of a 30-year-old with a work contract working in wet conditions for more than 5 hours would have higher predicted log cotinine values for any value of smoking. Generally, for smokers who smoke more than a few cigarettes per day in the reported week, the risk of additional cotinine absorption from certain work tasks diminishes. Furthermore, for smokers who smoke more than about six cigarettes per day, there is little difference in the risks associated with different tasks.

Table 3. Survey Linear Regression Coefficients for Logarithm of Cotinine

	Estimate	SE	*P* Value
Intercept	0.741	0.360	0.047
Age	0.013	0.005	0.007
Work contract	0.252	0.108	0.025
Primary type of work (reference: did not work)			
Prime	3.255	0.363	<0.001
Prime/barn	2.966	0.356	<0.001
Barn	1.122	0.357	0.003
Top	1.830	0.369	<0.001
Other	1.451	0.615	0.024
Season (reference: late)			
Early	-1.275	0.290	<0.00
Middle	-0.583	0.101	<0.001
Work conditions (reference: dry)			
In wet clothes >5 hrs	0.282	0.144	0.058
In wet clothes <5 hrs	0.100	0.133	0.457
Ln (1 + cigarettes smoked) (no reference group)			
Prime or prime/barn	0.311	0.079	<0.001
Barn	0.814	0.177	<0.001
Top	1.187	0.092	<0.001
Other	1.234	0.198	<0.001
Did not work	3.474	0.626	<0.001

	Estimate	SE	*P* Value
Ln (1 + cigarettes smoked)^2			
Did not work	-0.675	0.253	0.011

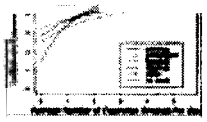

Fig. 2. The relationship between number of cigarettes smoked and log salivary cotinine for the late season in the context of different types of tobacco work for **farmworkers** in North Carolina, 1999. Curves are for fitted values for a 30-year-old worker, with work contract, working in dry conditions.

All additional two-way interactions involving smoking were non-significant, with one exception. There was an interaction of work type and season primarily attributable to an unusually high mean cotinine level for the "other" work combined with an unusually low mean cotinine level for "did not work" at mid-season. We chose not to model this "local" interaction because its inclusion did not qualitatively affect the overall results.

Discussion

Smoking status, as defined in this analysis, seems to be confirmed by the cotinine analysis. Values were lowest in the non-smokers and highest in those who always smoked, with occasional smokers falling (as expected) between these groups. The early-season values, when plant nicotine exposures should have been low, placed non-smokers' cotinine levels within the range usually found for non-smokers. The mean early-season cotinine level of 104 ng/mL for the AS group is relatively low for US active smokers. Although Hispanic smokers tend to have somewhat lower serum cotinine levels in general, after adjusting for the serum-saliva relationship as previously described, [13] the concentration in the AS group remained substantially below the expected mean salivary cotinine value of about 156 ng/mL for Mexican-American smokers in the US population. [15] However, the number of cigarettes smoked by the AS group is quite low. The cotinine value in this group is consistent with Etter et al's finding of a 14-ng/mL saliva cotinine increase per

cigarette. [16]

Within each smoking status group, cotinine levels increased across the summer work periods. In late summer, cotinine levels in non-smokers were at a level similar to those of early-season smokers. The increase in cotinine level was similar in all smoking groups, approximately 80 ng/mL, which might be interpreted as reflecting the dose of nicotine received in the course of performing tobacco work. Experimental studies of transdermal nicotine absorption have found the rate of absorption lower in those with higher serum cotinine levels. [17] The multivariate analyses confirm this finding, indicating that there is a diminishing effect of the number of cigarettes smoked on raising the cotinine level higher when it is already high. Studies of green tobacco sickness and anecdotal reports from tobacco growers suggest that smoking is protective against this illness. [3] [4] [8] If this is the case, the mechanism of protection probably involves more than reduced transdermal absorption.

In the bivariate analyses of cotinine predictors, several work-related predictors were associated with cotinine, as expected. Types of work associated with the greatest exposure to wet plants and to mature plants with the highest nicotine content resulted in the highest cotinine levels. The greater the number of hours worked in tobacco, the higher the cotinine level. Wet work conditions were also associated with higher cotinine levels.

Several other predictors may be related to cotinine in directions opposite to that expected. Higher cotinine values were associated with changing out of wet clothing in all smoking groups, and with wearing a rain-suit in all but the AS group. This may reflect the greater likelihood of changing clothes and of wearing protective gear when conditions are at their wettest, which is also when the nicotine exposure from plants should be highest. Because both changing clothes and wearing rain-suits are inconvenient and interfere with work, we presume that workers do this only in extreme circumstances (eg, very wet conditions). Therefore, these behaviors represent nicotine exposure conditions more so than protective behaviors.

In this study, environmental tobacco smoke exposure through living with a smoker did not have a significant effect on cotinine levels across the whole sample, although a trend consistent with the expected association was seen for non-smokers during non-work periods. Smokers in the home are usually a significant source of environmental tobacco smoke exposure among non-smokers in the US population. For example, the geometric mean serum cotinine concentration among adults in The Third National Health and Examination Survey with no known home or workplace

exposure was 0.124 ng/mL; this increased to 0.700 ng/mL among those who were regularly exposed to environmental tobacco smoke at home. [12] Our failure to observe a similar response with salivary cotinine among tobacco workers in this study is probably largely attributable to the dominating effect of the work-related nicotine exposures in this population.

Our model explained 68% of the variation in log cotinine. This might have been higher had there been greater precision in the measurement of smoking exposure. Workers reported a single value for daily smoking in the previous week. Because the mean elimination half-life of serum nicotine is approximately 4 hours, [18] the day or two immediately before sample collection were likely to have the greatest effect on salivary cotinine. In addition, we made some assumptions in defining the primary type of work. For example, because specific hours for each task were not recorded for each workday, we created a category of priming/barning if both tasks were indicated on each of 2 days. Despite these assumptions, the model demonstrates the combined effects of work type, season, wet working conditions, and smoking status on cotinine levels.

Priming has the greatest effect on cotinine, approximately twice (on the log scale) that of topping and barning. This is probably because of the way flue-cured tobacco is handled by workers. Topping requires workers to break the flower from the plant, exposing the hand to the plant juices. Workers "prime" or harvest tobacco by bending over or under the plant to break off the mature leaves. Leaves are carried under the arm as workers move down the row of plants. When no more can be carried, the leaves are deposited on carts. As the leaves are carried, the axillary region becomes covered with the sticky plant juices. In studies of percutaneous chemical absorption, axillary skin has been shown to absorb 360% of the reference standard (ventral forearm). [19] Barning results in less exposure than priming, because workers use their hands and arms to transfer the leaves from carts to bulk barns. The palmar surface absorbs only 83% of the ventral forearm reference.

Two demographic variables, age and having a work contract, remain as significant predictors of cotinine in the multivariate model. These predictors may indicate intensity of work. Older workers, in general, are more experienced and may accomplish more of a given work task in the same hours than a younger and less experienced worker.

Conclusion

Most migrant workers hired for tobacco work today are from Mexico and have little or no prior experience with tobacco production. Thus, they are typically unaware of the nicotine exposure caused by their work. Although US Environmental Protection Agency regulations mandate worker training for pesticide safety, there are no regulations requiring employers to inform workers about exposures to nicotine. The results presented here are the first to demonstrate the effect of a high level of work-related nicotine exposure on cotinine levels among tobacco workers. The findings indicate that working in tobacco results in significant transdermal absorption of nicotine. Although the short-term effects of this exposure may be symptoms of nicotine poisoning (green tobacco sickness), the long-term effects of such exposure should be investigated.

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