Pesticide Exposures, Cholinesterase Depression, and Symptoms among North Corolina Migrant Farmworkers

Resource Id#: 5011

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ABSTRACT

Objectives. We conducted a clinic-based study of crythrocyte cholinesterase levels, pesticide exposures, and health effects among farmworkers and nonfarmworkers to determine risks for exposure and associated morbidity.

Methods. Two hundred two farmworkers and 42 nonfarmworkers were recruited sequentially at two community health centers. Erythrocyte cholinesterase levels were measured colorimetrically. Questionnaires obtained data on demographics, occupational history, exposures, and symptoms.

Results. Cholinesterase levels were significantly lower among farmworkers (30.28 U/g hemoglobin) than among nonfarmworkers (32.3 U/g hemoglobin). Twelve percent of farmworkers, but no nonfarmworkers, had very low levels. Farmworkers applying pesticides also had lower cholinesterase levels. One half of farmworkers reported being sprayed by pesticides and working in fields with an obvious chemical smell. Of reported symptoms, only diarrhea was associated with cholinesterase levels. Reported exposures, however, were strongly associated with symptoms.

Conclusions. Farmworkers reported many pesticide exposures that violate state and federal regulations. Farmworkers had cholinesterase levels significantly lower than those of nonfarmworkers, although only spraying pesticides was associated with very low levels. (Am J Public Health 1994;84:446-451) Stephen Ciesielski, PhD, MD, Dana P. Loomis, PhD, Susan Rupp Mims, MD, and Annella Auer, MPH

Introduction

Exposure to cholinesterase-inhibiting pesticides such as organophosphates and carbamates is considered a major health problem for the 2.5 to 5 million farmworkers in the United States and the 80 000 farmworkers in North Carolina.¹⁻⁶ Clinical manifestations of such exposure are extremely diverse.7-9 Longterm exposure has been implicated in several types of cancer, teratogenic effects, sterility, spontaneous abortion, and cognitive deficits.10,11 Major poisonings among farmworkers are fairly well characterized, but the health effects of routine, smaller exposures are uncertain. Previous studies, although limited by small sample size and inadequate comparison groups, revealed depressed cholinesterase levels in farmworkers, 12-16 the pathophysiological significance of which remains unclear. Thus, we conducted a clinic-based study of farmworkers and nonfarmworkers in North Carolina to assess pesticide exposure, the symptoms occurring among exposed workers, and the effect of simple measures to protect against exposure.

Methods

Subject Selection

Every third patient presenting to two community health centers was an eligible subject. Those performing farmwork for 1 week of the previous month (i.e., those with the potential for occupational exposure) were eligible for the farmworker group. Those who had never performed migrant farmwork were eligible for the unexposed nonfarmworker group. Two hundred two farmworkers and 42 nonfarmworkers participated; nonresponse was 4% (8) for farmworkers and 5% (2) for nonfarmworkers. There was no attrition. Informed consent was obtained from subjects in their preferred language.

Measurement of Symptoms and Pesticide Exposure

Pretested, verbally administered questionnaires obtained information about demographic characteristics, work history, occupational pesticide exposure within 1 month of enrollment, and symptoms within 1 week of enrollment. Nonfarmworkers were questioned about domestic and occupational exposure to pesticides. Because exposure to cholinesterase-inhibiting pesticide produces characteristic biologic effects,8-10 a fairly comprehensive range of symptoms was included in the questionnaire. Two symptoms thought not to be associated with exposure-increased nail friability and dysgeusia (altered taste perception)were also included to assess the extent of recall bias.

Erythrocyte Cholinesterase Measurement

Cholinesterase depression represents an unambiguous indication of exposure to cholinesterase-inhibiting pesti-

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This paper was accepted August 24, 1993.

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Pesticide Exposure

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Five microliters of blood were obtained by finger prick. Hemoglobin and acetylcholinesterase activity were measured with the Test-Mate OP Kit (EQM Inc, Junefield, Ohio). This field assay uses a modified Ellman method17 and has received extensive field and lab testing.18-21 Acetylcholinesterase activity, measured as absorbance, was corrected for ambient temperature and hemoglobin. Replicate tests were performed on subjects with acetylcholinesterase levels below 25 U/g hemoglobin. Field staff tested themselves daily as an external quality control. Subjects' results from days on which the operator's acetylcholinesterase level varied by more than 10% from the operator's mean were not included in the final analysis.

Symptomaticity may occur only with severe acetylcholinesterase depression.* Therefore, measurement of the frequency of adverse effects in a population may depend more strongly on the proportion with very low acetylcholinesterase levels than on those whose levels represent the population mean. To account for this possibility, cholinesterase levels were dichotomized into normal and low categories as well as treated on a continuous scale. The cutpoint for these categories was one standard deviation below the mean of the nonfarmworker group.

Data Analysis

Like many biochemical markers, acetylcholinesterase inhibition can be viewed as a measure of either exposure or outcome. In the first part of the analysis, employment as a migrant farmworker was taken as an indicator of the potential for pesticide exposure, cholinesterase levels were examined among farmworkers and the comparison group of nonfarmworkers, and their mean cholinesterase levels were compared. In addition, odds ratios were used to compare the observed and expected numbers of "cases" with extremely low cholinesterase levels among farmworkers, deriving the expected numbers from the experience of the nonfarmworker comparison group.

Subsequent analyses focused on the relationship of acetylcholinesterase depression among farmworkers to specific occupational and behavioral indicators of potential exposure; nonfarmworkers could not, by definition, have had these exposures. Acctylcholinesterase levels for farmworkers who reported a given exposure or activity were compared with levels for farmworkers without that factor, and the data were treated in both continuous and dichotomous forms as before.

The third and final part of the analysis sought to identify symptoms associated with exposure. Instances of workers who reported a given symptom were treated as cases. Pesticide exposure was defined by both an extremely low acetylcholinesterase level and reported occupational exposure through using pesticides, being sprayed, or working in recently sprayed areas. For each symp-

tom, odds ratios were used to estimate the ratio of observed to expected cases.

The statistical analysis was performed using the SAS system (SAS Institute, Cary, NC). Coefficients for linear regression models were estimated by least squares, with P values derived from standard F tests. Confidence intervals (CIs) for crude odds ratios were based on Woolf's method, and the Mantel-Haenszel chi-squared statistics were used for hypothesis testing.22 Adjusted odds ratios were estimated by logistic regression. A CAR WAR

Results

Both groups were demographically similar with regard to birthplace, but farmworker subjects were younger and more often male (Table 1),

Figure 1 shows the distributions of cholinesterase levels between the two groups. The mean acetylcholinesterase level of the farmworkers (30.18 U/g hemoglobin) was significantly lower than that of the nonfarmworkers (32.20 U/g hemoglobin; P = .01) (Table 2). No nonfarmworkers had acetylcholinesterase levels below 25.28 U/g hemoglohin (the lower limit of the nonfarmworker mean minus one standard deviation), while 24 (12%) of the farmworkers did (P = .019).

Many farmworkers reported occupational exposure to pesticides. One type of exposure derives from the application of pesticides. Forty-seven percent of

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	Farmworkers			N	onfarmwo		
	No.	Mean Value	No. Cases ^a	No.	Mean Value	No. Cases	Difference in Means
Ethnic group							
US-born Blacks	69	31.00	8	11	32.29	Ó	13
Hispanics	120	29.73	13	21	32.17	ō	2.4*
Otherb	13	29.97	3	10	32.18	õ	2.2
All subjects	202	30.18	24	42	32.20	Ø	2.1*

*Those with cholinesterase levels below 25.28 U/g hemoglobin, the lower limit of nonfarmworkers (those unexposed) minus one standard deviation.
*Haitfans, Whites, Puerto Ricans, Jamalcans.

*P < .05. Student's t test.

P < .05, Student's ritest.

farmworkers reported being sprayed directly with pesticides or working immediately adjacent to rig spraying within 1 month prior to study enrollment (Table 3); 33% of these subjects reported subsequent illness. Fifty-one percent of the subjects reported working in fields with an obvious chemical smell. None of these reported exposures was associated with significantly lower acetylcholinesterase levels although among those reporting exposures was a consistently greater proportion of cases with acetylcholinesterase below 25.28 U/g hemoglobin.

In contrast, the mean acetylcholinesterase level of farmworkers applying pesticides was 2.7 U/g hemoglobin lower than that of other farmworkers (P = .002), and the odds ratio for the association of having extremely low acetylcholinesterase with applying pesticides was 4.2 (95% CI = 1.1, 16) (Table 3). There were no significant differences among the mean acetylcholinesterase levels of farmworkers employed in different crops.

A second type of exposure derives from pesticide residues and relates to potentially protective behaviors such as wearing gloves or washing hands (Table 4). Low acetylcholinesterase levels had little or no association with these exposures. The majority of subjects (58%) reported that handwashing water was not consistently available. Similar analyses that used linear and logistic regression models and adjusted for age, years in farmwork, demographic characteristics, and multiple exposures produced essentially the same results.

When the frequency of symptoms among farmworkers was examined with respect to acetylcholinesterase levels below and above 25.28 U/g hemoglobin, only diatrhea was substantially associated with lower levels (odds ratio = 3.1; 95% CI = 1.18, 8.0) (Table 5). However, 10 of the 16 symptoms had positive associations with reported exposure. Neither of the variables included to assess possible recall bias was associated with acetylcholinesterase levels, but dysgeusia did have an association of borderline significance with reported exposure (Table 5).

Discussion

To our knowledge, this is the largest epidemiological study to date of the health effects on farmworkers of exposure to cholinesterase-inhibiting pesticides. A clinic-based design was chosen to maximize inclusion of morbidity in order to examine the biologic relation between illness and acetylcholinesterase levels. The methodological difficulties in the epidemiological study of the health of migrant farmworkers relate primarily to worker transience and workplace conditions. Attrition complicates longitudinal study: in previous research, 74% to 83% of subjects were lost to observation within 1 to 3 years.^{23,24} In cross-sectional studies, constraints on determining etiology are compounded by barriers to observational data collection and by the fact that workplace exposure and conditions may change daily.

Reliance on reported exposures poses obvious problems. Most farmworkers do not know to what pesticides they may be exposed. Moreover, although we saw no evidence of it, recall bias may cause overreporting of exposure among symptomatic subjects. Underreporting may be more common; state and federal regulations prohibit fieldwork before sprays have dried or dusts have settled,²⁵ but perceptible signs of reentry time violation may not exist. An obvious chemical smell indicates that pesticides remain suspended or liquid. Fifty percent of subjects reported exposure both to pesticide suspensions and to aerial or rig spraying, and one third of these subjects reported subsequent illness. Even allowing for substantial misattribution, it appears that occupational exposures to pesticides may routinely result in illness among farmworkers.

The most significant associations between occupational conditions and low acetylcholinesterase levels were demonstrated for workers applying pesticides. North Carolina regulations require that all individuals involved in the application of pesticides receive training and be licensed. No subjects who worked applying pesticides and who had low acetylcholinesterase levels were licensed.

The prevalence of protective behaviors was higher for those actions directly under the workers' control, such as the use of protective clothing. However, even individual protective behaviors such as washing are limited by occupational conditions such as the availability of water. Subjects' motivation to engage in protective behavior is indicated by the fact that as many subjects reported handwashing before eating as reported the availability of handwashing water. The unavailability of handwashing water (reported by 58%) is exactly the same as that obtained by a study conducted several years earlier,²³ suggesting that, despite promulgation of new field sanitation laws, lack of enforcement allows unsafe working conditions to continue.

Associations between exposure and low acetylcholinesterase levels can be examined without knowing the specific pesticides involved because no other significant environmental exposures lower erythrocyte cholinesterase.26 As did earlier studies, 12-16 we found that farmworkers have lower acetylcholinesterase levels than nonfarmworkers. The difference in means was statistically significant, but its biologic meaning is uncertain. Of more probable importance is that 12% of farmworkers had levels below the lower limit of the nonfarmworker group. Since the nonfarmworkers were not really unexposed (all lived in heavily sprayed agricultural areas and many reported using pesticides on gardens or pets), the difference between a farmworker group and an unexposed group may be underestimated.



TABLE 3-Cholinesterase Levels and Occupational Exposures to Pesticide Application Reported by Farmworkers* - 2 33

	Farmw	orkers	Difference in Means of Erythrocyte	Cases ^e Exp	Cases ^e among Exposed		Cases among Unexposed		
Exposure Variables Belated to Pesticide Application	No.	%	Cholinesterase Level ^b	No.	%	No.	%	Odds Ratio	95% Cl
Being sprayed directly or indirectly Working in fields with chemical odor Mixing or applying pesticides	92 99 9	47 51 06	0.1 0.1 -2.7	13 13 3	14,1 13.1 33.3	11 11 1	10.5 11.6 10.5	1.4 1.2 4.2	

"Reported exposures within the 30 days prior to interview.

"Difference between the means of the exposed and unexposed groups.

Subjects with acetylcholinesterase below 25.28 U/g hemoglobin.

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The health effects investigated all have multiple causes, many especially prominent in farmwork. Rashes result from plant allergens, dermatoses, sun, heat, infectious causes, and a variety of pesticides. Interactions with other health risks in farmwork may not only complicate diagnosis and study but also place farmworkers at greater risk. For example, dermatoses increase absorption of pesticides, and dehydration and poor nutrition increase their toxic effects.

Only minimal association was observed between low acetylcholinesterase levels and morbidity. However, a moderately strong association was observed with diarrhea, reported as one of the most common effects of such exposure.²⁷ Several explanations for this relative lack of association exist:

 No morbidity resulted because acetylcholinesterase levels were not sufficiently depressed to cause appreciable illness. It is also possible that tolerance develops to acetylcholine or that production of acetylcholinesterase increases in the face of repeated exposure.²⁶

 Limitations of cross-sectional study design introduced time-related misclassification of exposure and/or outcomes. Collecting data on exposures in the previous month but on symptoms only during the previous week may have allowed acetylcholinesterase depression to continue but associated symptoms to resolve.

3. Changing exposure to cholinesterase-inhibiting pesticides and other pesticides causing similar symptoms by different mechanisms may obscure the relationship between exposure and illness. Also, since carbamates reversibly inhibit acetylcholinesterase, resolution of previously depressed acetylcholinesterase levels may have occurred before enrollment. TABLE 4—Cholinesterase Levels and Occupational Exposures to Pesticide Residues Reported by Farmworkers*

	Exposure Variables	Farmwo	orkers	Cases ^b Expo	among sed	Cases a Unexp	among iosed	Odda	an a
	to Pesticide Residues	No.	%	No.	%	No.	%	Ratio	95% CI
	Aggregate of expo- sures below	129	66	16	12	8	12	1.0	.42, 2.6
	Did not always wear Shirt Long pants Shoes Long-sleeved shirt Gloves	28 35 * 41 113 165	18 18 21 57 83	3 3 5 16 20	11 9 12 14 12	18 21 19 8 4	14 13 12 9 12	.73 .64 1.0 1.6 1.0	.20, 2.6* .18, 2.3 .36, 2.9 .67, 4.0 .32, 3.2
	Handwashing water not always available ^e	116	58	14	12	10	12	1.0	.43, 2.4
Constability	Did not always wash hands Before eating in fields Before smoking in fields ^d Before excretory activity	73 91 173	50 96 87	9 8 20	12 9 12	8. 8. 0. 0. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1.	1	1.1 .92 .76	.41, 3.1 .04, 18.5 .24, 2.4
	Did not always launder clothing before wearing again	7.	38	10	13	5. 14	87) - 11	12	.49, 2.7
	Did not always wash harvested produce before consumption	25-1 51	کری 50	4. 	8	5 5 7 9	18	.38	.12, 1.3

Reported exposures within the 30 days prior to interview.

Subjects with enythrocyte cholinesterase levels below 25.28 U/g hemoglobin.

SAvailability of handwashing water does not relate directly to residue exposure but directly limits the ability of individuals to accomplish protective behaviors related to the use of water for washing. *For those who smoke.

Reported exposures, however, were strongly associated with reported symptoms. The results of the symptom variables included to assess recall bias suggest that such bias was minimal. Farmworkers who reported exposures were no more likely to report nail friability than those who did not, although dysgeusia was of borderline significance. However, dysgeusia may be indirectly related to exposure since it may result from sialorrhea or pausea.²⁸

Given the evidence of exposures in excess of those permitted by federal regulations, of the depression of acetylcholinesterase levels, and of the associa-

	Low Acety	Icholinesterase	Reported Exposure		
	OR	95% CI	OR	95% Cl	
Symptom/sign					
Diarrhea	3.1	1.18.8.0	8.2	2.3. 29.2	
Nausea	1.8	0.72.4.2	2.8	1.4.6.1	
Rash	1.5	0.63, 1.9	2.7	1.4. 5.2	
Red/irritated eyes	1.5	0.59. 3.6	1.4	0.73. 2.7	
Fever	1.6	0.53, 4.2	1.3	0.61.2.7	
Increased sweating	1.4	0.56. 3.4	1.5	0.76. 2.8	
Increased anxiety	1.1	0.43. 2.8	2.1	1.5.6.4	
Dizziness	1.2	0.48, 2.9	2.1	1.0, 4.1	
Headache	0.91	0.39, 2.1	2.2	1.2.3.9	
Blurred vision	1.4	0.56. 3.4	2.3	1.1.4.8	
Muscular symptoms ^d	0.98	0.41, 2.3	2.8	1.5.5.2	
Chest pain	0.99	0.38, 2.6	4.7	1.7. 13.1	
Sialorrhea	0.98	0.31.3.1	1.3	0.56. 2.8	
Difficulty breathing	0.36	0.12, 1.3	1.8	0.87.3.7	
Ataxia	0.55	0.12.2.4	5.0	1.6. 15.7	
Memory loss	0.36	0.05, 2.6	2.9	.87, 10.1	
/ariables to assess recall bias					
Dysgeusia	1.3	0.43, 3.6	2.5	1.0.6.0	
Friable nails	0.32	0.05. 2.2	1.9	0.67.5.3	

TABLE 5—Associations between Reported Symptoms and Low Erythrocyte Cholinesterase Values^a and Reported Pesticide Exposures^b

*Low erythrocyte cholinesterase defined as below 25.28 U/g hemoglobin.

Category of those exposed includes subjects reporting one of the following: (1) being sprayed directly. (2) working in fields near spraying, (3) working in fields with a chemical smell, or (4) mixing/applying pesticides.

Crude odds ratios were obtained from Mantel-Haenszel chi-squared statistics.

Muscular disorder includes weakness, muscle cramps, and muscle fasciculations.

tions between exposure and reported symptoms, it seems probable that morbidity results from these more routine types of pesticide exposure among farmworkers despite the relative lack of association with acetvlcholinesterase depression. It is also possible that the cross-sectional nature of this study limited the identification of morbid sequelae. This conclusion is strengthened by anecdotal experience. The same acetylcholinesterase field assay was later used to diagnose pesticide-related illness in a farmworker clinic. Seventeen individuals with characteristic symptoms were tested; four had acetylcholinesterase levels below the lower limit of normal. Their acetylcholinesterase levels had increased for 3 to 4 successive weeks before the subjects were lost to follow-up; however, their symptoms had usually resolved within 1 week of exposure.

It is important that additional research be undertaken for several reasons. First, migrant health programs have begun to dedicate much of their extremely limited funds to pesticide issues despite uncertainty about the extent of health effects. Most funding goes to educational programs designed to change farmworker behaviors, although exposures leading to serious illness or death are not likely to be under workers' control. Second, this study only addresses acute and subacute neurotoxicity. Thus, the relationship between organophosphate and carbamate exposure and other serious health effects such as cancer remains controversial even though such outcomes may be the most significant health effect of pesticide exposure.¹¹ Cholinesterase testing may eventually serve a more important role in monitoring workers for long-term cancer risk than for short-term neurotoxicity.

Paradoxically, although pesticides are routinely regarded as a serious health risk for farmworkers, few if any migrant health clinics are capable, in terms of technology, diagnostic protocols, and logistics, of diagnosing pesticide-related illness. We strongly recommend that migrant health centers develop means of routine cholinesterase testing to improve both diagnosis of pesticide poisoning and descriptions of the nature and extent of pesticide-related illness among migrant farmworkers on a population level.

Although population parameters cannot be estimated, some conclusions can nevertheless be drawn from these results. First, many patients presenting to farmworker clinics may have had recent pesticide exposures exceeding those permitted by federal regulations. These patients often believe that such exposures have adversely affected their health. Second, depressed acetylcholinesterase levels are more often found among farmworker patients than among nonfarmworker patients. The clinical significance of these low acetylcholinesterase levels cannot be determined on the basis of these data. Finally, farmworkers' behavior suggests that they are motivated to reduce pesticide exposures but that their ability to do so is constrained by occupational conditions beyond their control.

Acknowledgments

Funding for this study was provided by the North Carolina Primary Health Care Association (NCPHCA) in Cary, NC. Gratitude is expressed to Susan Sparrow, NCPHCA migrant specialist, to Noni Estelli for assistance with field work, to the Tri-County Community Health Center in Newton Grove, NC, and to Goshen Medical Center in Faison, NC.

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