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Neurological Symptoms Among Sri Lankan Farmers Occupationally Exposed to Acetylcholinesterase-Inhibiting Insecticides

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Background *In many agricultural districts in Sri Lanka, pesticide poisoning is a leading cause of death. This study aims to evaluate the impact of pesticide use on Sri Lankan farmers' health.*

Methods *A total of 260 subjects were surveyed in both a low and a high exposure period. Acetylcholinesterase activity was measured and data on symptoms were collected with questionnaires.*

Results *Twenty-four percent of surveyed farmers had suffered at least once from acute pesticide poisoning. Farmers showed significantly more inhibition of cholinesterase activity than controls. Acute symptoms indicative for exposure to cholinesterase-inhibiting pesticides were associated with farming and a higher degree of cholinesterase suppression (more than 13% inhibition). Integrated Pest Management (IPM) training seemed to result in less insecticide use, and less cholinesterase inhibition.*

Conclusions *Our results suggest that occupational acetylcholinesterase-inhibiting insecticide exposures have a negative impact on Sri Lankan farmers' health. Overall reduction in pesticide use seems the best option to protect farmers from the adverse effects of pesticides.* Am. J. Ind. Med. 44:254–264, 2003. © *2003 Wiley-Liss, Inc.*

KEY WORDS: pesticide exposure; acetylcholinesterase inhibition; neurological symptoms; IPM; Sri Lanka

INTRODUCTION

Pesticide poisoning is a major health problem in Sri Lanka [Jeyaratnam et al., 1982; Eddleston et al., 1998; Van der Hoek et al., 1998; Fernando and Hewagalage, 1999]. In many agricultural districts, pesticide poisoning is the leading cause of death [Ministry of Health, 2001]. Most clinically documented acute poisoning cases in Sri Lanka are deliberate (suicide) and occur among young adults [Van der Hoek et al., 1998]. Pesticide poisoning due to occupational exposure is poorly documented but is assumed to be common. One report claims that yearly five out of every 1,000 agricultural workers in Sri Lanka are hospitalized due to pesticide poisoning of occupational origin [Jeyaratnam et al., 1982]. However, many additional, less severe, cases of occupational poisoning may not require hospitalization and are, therefore, not included in the total cases reported.

Since the 1980s, organophosphate compounds have been the principal means of agricultural pest control throughout the world [Stephens et al., 1995]. Organophosphates and *N*-methyl carbamate insecticides have well-documented acute systemic effects largely mediated through cholinesterase inhibition leading to an overstimulation and then

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depression of the nervous system [Yuknavage et al., 1997]. Early symptoms of acute poisoning include weakness, nausea, vomiting, excessive sweating and salivation, headache, and difficulty in walking [Namba, 1971]. Severe poisoning may result in unconsciousness, pulmonary edema, respiratory failure, and death [Al-Shatti et al., 1997]. Single episodes of clinically significant organophosphate intoxication are associated with a persistent decline in neuropsychological functioning [Rosenstock et al., 1991]. Long-term, relatively low exposure to pesticides, particularly organophosphate insecticides, is increasingly suspected of causing similar effects on the nervous system [Stephens et al., 1995; Wesseling et al., 1997; London et al., 1998]. However, firm conclusions on neuropsychological effects of chronic exposure to pesticides are difficult to draw as information is scarce, particularly in developing countries [London et al., 1998; Tinoco-Ojanguren and Halperin, 1998; Maroni et al., 1999].

Farmers in Sri Lanka have knowledge of the existence of, and limited access to, personal protective devices when applying pesticides, although they rarely apply them. Protective devices are found to interfere with work practices, are uncomfortable in the hot, humid climate, and are time consuming to put on [Sivayoganathan et al., 1995; Van der Hoek et al., 1998]. In addition, the effectiveness of these devices may be inadequate due to penetration of pesticides through clothing and occlusion of exposed skin by gloves and clothing [Van Wendel de Joode et al., 1996; Meuling et al., 1997; Spruit and Van Puyvelde, 1998; Brouwer et al., 2000]. Van der Hoek et al. [1998] proposed alternative ecological methods of pest control within the context of Integrated Pest Management (IPM) as a strategy to reduce pesticide use in Sri Lanka. IPM makes full use of natural and cultural pest control processes and methods, and also involves a wide range of other practices aimed at growing a healthy crop. Farmers learn about the ecology of their fields and, as a result, they make and implement decisions, which are safe, productive, and sustainable. Chemical pesticides are used only where and when the above measures fail to keep pests below damaging levels [FAO Programme for Community IPM in Asia, 2001].

This study was part of an ongoing research project of the International Water Management Institute (IWMI) in the Uda Walawe Irrigation Scheme in southern Sri Lanka. The study was undertaken to evaluate the impact of different pesticide usage patterns on the health of farming families in the Uda Walawe Irrigation Scheme. The aims of study were: to assess (neurological) symptom prevalence in relation to occupational exposure to cholinesterase-inhibiting pesticides; to determine the extent of acetylcholinesterase inhibition in general farmers and IPM-farmers in a high exposure period compared with a control group; to investigate relationships between symptoms, acetylcholinesterase inhibition, and cholinesterase-inhibiting pesticide exposure.

SUBJECTS AND METHODS

Study Population

The study population consisted initially of 242 farmers (all smallholders), including 131 farmers trained in using IPM in paddy (unhusked rice) cultivation. IPM-farmers were recruited by selecting randomly from a list of farmers who had attended IPM demonstrations conducted by the Mahaweli Authority of Sri Lanka in the Uda Walawe Irrigation Scheme. Other farmers were matched within the same villages. A control group of 55 fishermen was recruited from a fishing community of the Uda Walawe reservoir. Of 55 controls, 27 had previously cultivated crops, one or more years ago (average time since cultivation was 8.1 years). Thirteen controls reported having household members who spray pesticides.

Data Collection

Data were collected with questionnaires and by measuring red blood cell acetylcholinesterase activity in two different periods (April–May 2000 and June–July 2000). The first period was in-between *Maha* (October to March) and *Yala* (May to August), the two agricultural seasons in Sri Lanka. This was presumably a low exposure period, and cholinesterase activity levels measured during this period were considered baseline values. A second round of data collection took place during *Yala* agricultural season, a high exposure period. Recruited farmers were requested to come to a central location in their village on a scheduled day to participate in the study. The rate of follow-up was 84.7% of the baseline population for farmers ($n = 94$), 93.1% for farmers trained in the use of IPM ($n = 122$), and 80.0% for controls ($n = 44$).

For each cholinesterase test 10 μl of blood were obtained from a finger-prick sample. Erythrocyte acetylcholinesterase activity in whole blood and hemoglobin were measured with the Test-mate ChE Cholinesterase Test System (EQM Research, Inc., Cincinnati, OH). This World Health Organization (WHO) approved field kit is based on the method of Ellman et al. [1961]. Acetylcholinesterase activity is expressed in units (U)/g hemoglobin. The acetylcholinesterase activity in the high exposure period divided by the activity in the baseline period represents the change in acetylcholinesterase activity within an individual ([1 − change in acetylcholinesterase activity] × 100% = acetylcholinesterase inhibition).

Structured, pre-tested, questionnaires were used to obtain information on personal characteristics and pesticide handling. Subjects were asked questions on age, educational level, tobacco and alcohol consumption, IPM training, spray activities during the previous month, cropping details, pesticide expenditure, and spray activities during the entire

Maha season (the previous season), use of protective measures and previous acute pesticide poisoning. The quetelet index (weight/square height (kg/m^2)) was calculated to determine the nutritional status of the subjects. A trained interviewer administered the questionnaires.

Furthermore two questionnaires were used to assess neurological symptoms. One of the two questionnaires was compiled of published signs and symptoms considered indicative for exposure to organophosphate and carbamate pesticides and assessed the prevalence of 16 acute symptoms experienced in the previous week [Namba, 1971; Derogatis and Spencer, 1982; Xue, 1987; Rosenstock et al., 1991; Rola and Pingali, 1993; Kishi et al., 1995; London et al., 1998]. Earache was added as a dummy symptom to control for bias. The second questionnaire consisted of 16 neurological symptoms derived from a previously validated Swedish 16-item questionnaire (Q16) [Hogstedt et al., 1984; Lundberg et al., 1997]. These symptoms are considered indicative for long-term health effects (early neurological disorders) due to insecticide exposure. Three questions were adapted, as they were clearly not applicable to the Sri Lankan situation: 'do you often make notes about what you must remember?' and 'do you generally find it hard to get the meaning from reading newspapers and books' were rephrased because many farmers were illiterate, 'do you have problems with buttoning and unbuttoning' was modified because most farmers usually wear clothes without buttons. Constipation was used as dummy symptom. Questionnaires were translated into Sinhalese and back-translated into English by a different translator to check whether questions had been translated correctly. Questionnaires were tested in a pilot-study among 20 farmers. Ethical clearance of the study was obtained from the Ethical Committee, Faculty of Medical Sciences, University of Sri Jayewardenapura, Sri Lanka. Informed consent was obtained from all study subjects. Subjects who had any symptoms of poisoning were referred to the Embilipitiya Base Hospital for further treatment.

Data Analysis

Data from subjects who had participated in both surveys (baseline and *Yala* season) was analyzed with SPSS (version 8.0). Acetylcholinesterase activity and inhibition levels were compared using analysis of variance (ANOVA). Cox's proportional hazard regression was used to calculate symptom prevalence ratios adjusted for potentially confounding variables [Skov et al., 1998]. Additionally, outcomes of the symptom questionnaires were dichotomized at more than six symptoms according to Hogstedt et al. [1984] (Q16), and at the 75th percentile of the study population (acute neurological symptoms). Acute symptoms significantly related to cholinesterase inhibition were dichotomized as well, using a positive score for more than three symptoms as a cut off point (75 percentile). The effects of confounding variables on test outcomes of symptom prevalence for Cox's regression modeling were studied. Variables that changed the model meaningfully (entering the model with P-value $<$ 0.20 and changing the prevalence ratio at least 5%) were kept. Results were adjusted for effects of age and years of education. Adjustment for sex, alcohol consumption, smoking habits, positive score for dummy symptoms, and acute pesticide poisoning in the past did not change results.

RESULTS

Table I shows personal characteristics of the study population. Some major differences were seen within the study groups of farmers, IPM-farmers and controls. No differences were observed between subjects available for follow-up and those who dropped out, with regard to personal characteristics, reported symptoms, and baseline acetylcholinesterase activity. IPM-farmers had received more frequent training in safe use of pesticides than other farmers had. IPM-farmers wore significantly more often headgear, long sleeved shirts, and long pants or a sarong during work in the field (chi-square, $P < 0.05$) and ate and drank less often during pesticide application (chi-square, $P < 0.005$). Personal protective equipment such as spectacles, footwear, gloves, or masks were rarely used in both groups of farmers.

Of all 216 farmers, 52 (24%) reported having suffered at least once from an acute occupational pesticide poisoning in the past (Table II). IPM-farmers who reported an acute poisoning episode had more often sought medical treatment (visited a doctor or hospital) than general farmers (84 vs. 52%). Frequently reported symptoms included fainting or unconsciousness, vomiting, nausea, blurred, or lost vision, headache, and dizziness.

A total of 514 spray operations (151 during the low exposure season and 363 during the high exposure season) were reported (see Appendix A). General farmers sprayed insecticides more often than IPM-farmers (71.3 vs. 53.7% of all spray operations). Chlorpyrifos (an organophosphate insecticide) was the most frequently used pesticide amongst both groups of farmers. Many farmers had problems recalling names of pesticides they had used in the previous month. Data on average spraying time per hectare of cultivated agricultural land in the preceding *Maha* agricultural season (October to March 1999/2000) are summarized in Table III. According to these data IPM-farmers spent considerably less time per hectare on spraying insecticides than general farmers (10.9 vs. 58.9 hr; Mann–Whitney test; $P < 0.001$). Much more time per hectare was spent on spraying insecticides on vegetables than on paddy and banana. The time spent on spraying herbicides was similar for both groups of farmers. The median expenditure on pesticides in the *Maha* season was 5,000 Rupees by general farmers and 3,000 Rupees by IPM-farmers (1 US$ = 80 Rupees).

TABLE I. Personal Characteristics of the Study Population of Sri Lankan Farmers, IPM-Farmers, and Fishermen (Controls)

	General farmers (n = 94)	IPM-farmers (n = 122)	Controls (n = 44)
Mean age in years (SD)*	38.4 (11.8)	50.5 (11.9)	34.8 (10.1)
Sex (% male)	94.7	87.7	97.7
Mean weight in kg (SD)*	49.7 (6.7)	51.6 (8.2)	56.0 (8.9)
Mean height in cm (SD)*	161.9 (7.8)	160.1 (7.5)	163.5 (6.6)
Mean quetelet index (SD)[a]*	19.0 (2.5)	20.1 (2.7)	20.9 (2.8)
Mean education in years school (SD)	6.3 (3.6)	6.9 (3.5)	6.0 (3.1)
Smoking (%)**			
Never smoked	30.9	29.5	15.9
Previous smoker	23.4	27.9	18.2
Current smoker	45.7	42.6	65.9
Alcohol use (%)**			
Never	23.4	31.1	15.9
Previous	24.5	13.9	11.4
Current	52.1	54.9	72.7
Received training in safe use of pesticides (%)**	4.3	39.3	
Use of protective clothing (%)			
Spectacles	0.0	0.8	
Footwear	1.1	0.0	
Gloves	3.2	2.5	
Nose/mouth covering mask	9.6	9.0	
Headgear**	57.4	78.7	
Long-sleeved shirt**	78.7	91.0	
Long pants/sarong**	89.4	96.7	
Did never smoke during pesticide application (% of smokers)	74.4	71.2	
Did never eat or drink during pesticide application (%)***	60.6	77.9	

SD, standard deviation; IPM, Integrated Pest Management.
*$P < 0.05$, ANOVA.
**$P < 0.05$, chi-square.
***$P < 0.005$ chi-square.
[a] Weight/square height (kg/m^2).

TABLE II. Self-Reported Acute Occupational Pesticide Poisoning in the Past Among General Farmers (n = 94) and IPM-Farmers (n = 122) in Sri Lanka

	General farmers (%)	IPM-farmers (%)
Had suffered at least once from an acute occupational pesticide poisoning in the past	27 (29)	25 (21)
Had suffered at least once from an acute occupational pesticide poisoning in the past (organophosphate or carbamate symptoms)	16 (17)	24 (20)
Had received medical treatment (% of farmers who had suffered from poisoning)	14 (52)	21 (84)
Had applied some form of self-treatment (% of farmers who had suffered from poisoning)	6 (22)	4 (16)
Had not sought any treatment (% of farmers who had suffered from poisoning)	7 (26)	0 (0)

TABLE III. Average Pesticide Spraying Time per Hectare on Different Crops During the Entire *Maha* Agricultural Season for General Farmers (no IPM) and IPM-Farmers in Sri Lanka

	Mean hectares cultivated in *Maha* (SD)	Average time of spraying per hectare during *Maha* (hours)		
		Herbicides	Insecticides	Fungicides
All crops				
No IPM (n = 94)	1.00 (0.55)	11.5	58.9	4.7
IPM (n = 122)	0.93 (0.39)	10.0	10.9	2.2
Paddy				
No IPM (n = 54)	0.50 (0.31)	14.9	35.6	2.3
IPM (n = 118)	0.67 (0.36)	13.6	10.8	3.8
Banana				
No IPM (n = 63)	0.43 (0.32)	10.4	12.3	0.2
IPM (n = 71)	0.32 (0.21)	8.5	4.9	0
Vegetables				
No IPM (n = 76)	0.53 (0.39)	10.8	136.9	10.6
IPM (n = 44)	0.26 (0.19)	7.3	45.9	0.5

Table IV summarizes mean levels of hemoglobin corrected acetylcholinesterase activity for farmers and controls during low and high exposure seasons as well as acetylcholinesterase inhibition levels. Data were normally distributed. Surprisingly, during the low exposure period (considered baseline levels) the control group showed lower average cholinesterase levels than both groups of farmers (ANOVA, $P < 0.05$). However, average cholinesterase inhibition during the high exposure season was significantly higher for both groups of farmers (ANOVA, $P < 0.001$) as compared to controls (3%). Average inhibition was higher in general farmers than in IPM-farmers (11 and 8%, respectively; Student's *t*-test, $P < 0.01$). No significant associations were found between cholinesterase inhibition and age, sex, education, smoking, and alcohol use.

Figure 1 shows the cumulative distribution of acetylcholinesterase inhibition among general farmers, IPM-farmers and controls. The order of the three distribution patterns shows that inhibition in general farmers was higher than in IPM-farmers while controls had the lowest inhibition. No inhibition above 27% was measured in any subject.

Figure 2 shows relationships between cholinesterase inhibition (>13% inhibition, 25 percentile) and determinants in farmers. Not having received IPM training and spraying insecticides more than 13 hr per hectare (median) during the entire *Maha* season were significantly associated with increased inhibition (Cox's regression, $P < 0.05$). Other factors such as not having received training in safe use of pesticides, previous poisoning, and not wearing a mask, headgear, or long pants showed weak positive associations with inhibition.

Adjusted prevalence ratios of 16 acute neurological symptoms experienced in the week before the interview during the high exposure period and sixteen Q16 symptoms are shown in Table V. Prevalence of six acute symptoms and two Q16 symptoms differed significantly between farmers

TABLE IV. Acetylcholinesterase Activities (U/g Hemoglobin) and Inhibition Among Sri Lankan Farmers, IPM-Farmers, and Fishermen (Controls)

	General farmers (n = 94)		IPM-farmers (n = 122)		Controls (n = 44)	
	Mean	SD	Mean	SD	Mean	SD
Acetylcholinesterase activity during low exposure period (baseline)	29.15	3.88	29.20	3.95	27.68	3.43
Acetylcholinesterase activity during high exposure period	25.97	3.42	26.76	3.35	26.79	3.75
Acetylcholinesterase inhibition (%)*,**	10.58	7.19	8.03	6.22	3.20	5.89

*$P < 0.001$, ANOVA.

**$P < 0.01$, farmers versus IPM-farmers, Student's *t*-test.

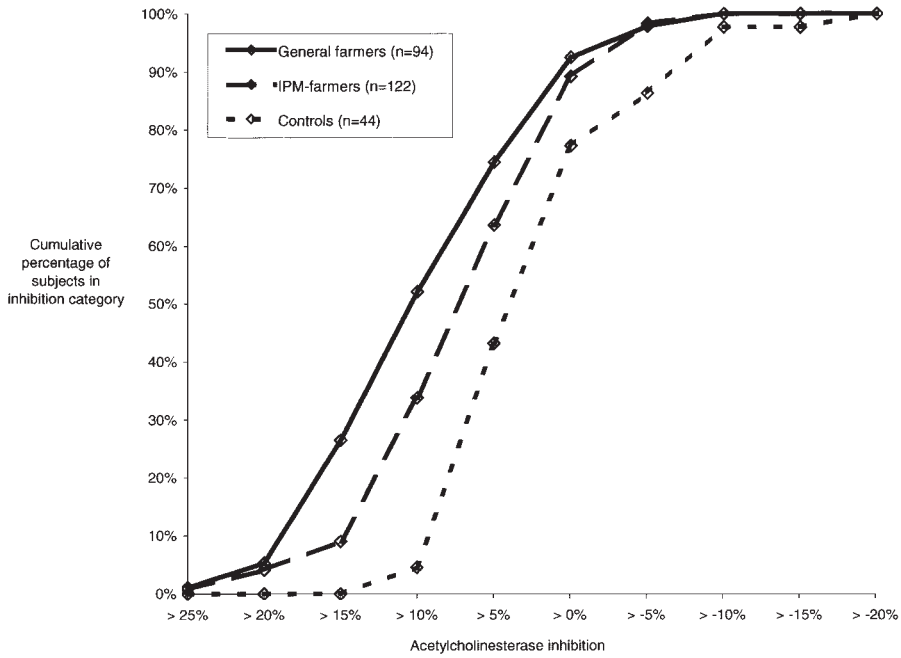

FIGURE 1. Cumulative distribution of acetylcholinesterase inhibition levels among general farmers, Integrated Pest Management (IPM)-farmers, and controls in Sri Lanka.

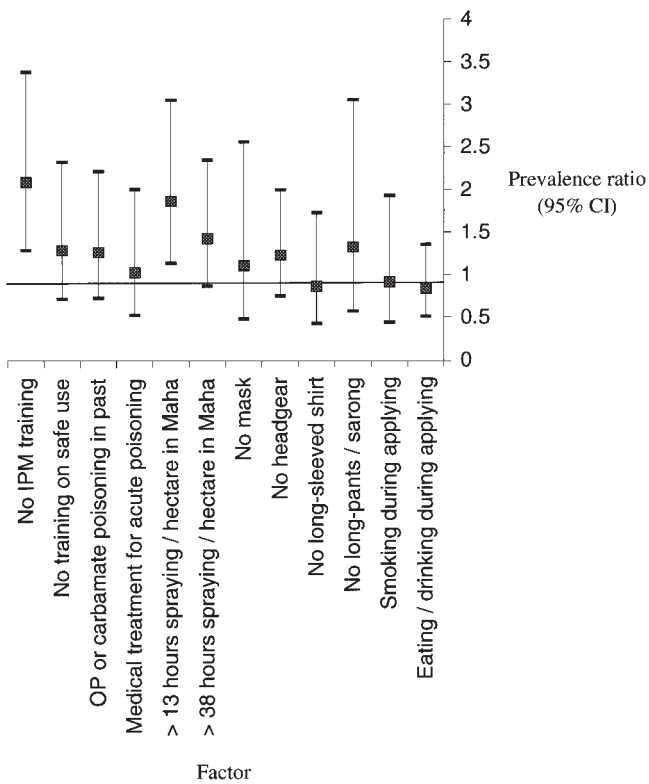

FIGURE 2. Factors possibly associated with increased acetylcholinesterase inhibition in Sri Lankan farmers (n = 216) (inhibition of ≥13% was used as cut off point).

and controls whereas prevalence of three acute symptoms and three Q16 symptoms differed significantly between IPM-farmers and controls (Cox's regression, $P < 0.05$). Further analysis did not reveal an obvious difference in symptom prevalence for general farmers and IPM-farmers: only one symptom (feelings of fear) was more prevalent among general farmers (the adjusted prevalence ratio (95% CI) was 2.33 (1.17–4.64). Prevalence of two dummy symptoms, not expected to be associated with pesticide exposure (earache and constipation) did not differ significantly between farmers, IPM-farmers and controls.

Seven of the sixteen acute symptoms (paleness, dizziness, muscle weakness, staggering, feelings of fear, tremor, and nausea) were significantly associated with increased cholinesterase inhibition (>13% inhibition, 25 percentile) after correction for age and education. The adjusted prevalence ratios (95% CI) were for paleness 1.50 (1.03–2.17); dizziness 1.58 (1.07–2.35); muscle weakness 1.67 (1.09–2.55); staggering 1.86 (1.23–2.80); feelings of fear 2.01 (1.16–3.48); tremor 1.74 (1.14–2.67); and for nausea 1.81 (1.02–3.20). The other acute symptoms, except diarrhea, were also positively associated with increased inhibition with prevalence ratios ranging from 1.05 to 1.73. Excessive perspiration was the only Q16 symptom significantly associated with increased inhibition (the adjusted PR (95% CI) was 1.59 (1.09–2.32). The other Q16 symptoms showed weak positive associations with increased inhibition, with prevalence ratios ranging from 1.06 to 1.45.

Table VI shows the adjusted prevalence ratios for dichotomized acute symptoms, acute symptoms associated with

TABLE V. Self-Reported Symptoms During High Exposure Period for General Farmers and IPM-Farmers in Sri Lanka With Control Group Used as Reference Category (n = 44)

	Adjusted prevalence ratios (95% CI)[a]	
Signs and symptoms	**General farmers (n = 94)**	**IPM-farmers (n = 122)**
Symptoms experienced during last week		
Excessive salivation*	6.56 (1.55–27.66)	3.70 (0.81–16.80)
Paleness *,**,***	4.35 (1.86–10.17)	4.49 (1.85–10.85)
Feelings of fear*,***	3.24 (1.25–8.45)	1.40 (0.47–4.22)
Staggering*,**,***	2.70 (1.13–6.46)	2.86 (1.16–7.05)
Dizziness*,**,***	2.63 (1.28–5.42)	2.18 (1.01–4.73)
Nausea***	2.59 (0.88–7.61)	2.20 (0.70–6.95)
Muscle weakness*,***	2.55 (1.13–5.77)	2.24 (0.95–5.31)
Nervousness	1.92 (0.98–3.76)	1.82 (0.88–3.76)
Numbness	1.61 (0.92–2.83)	1.69 (0.93–3.06)
Tremor[b]	1.51 (0.71–3.20)	1.81 (0.83–3.92)
Abdominal pain	1.50 (0.77–2.91)	1.22 (0.57–2.58)
Blurred vision	1.46 (0.87–2.47)	1.54 (0.89–2.67)
Muscle cramps	1.37 (0.66–2.85)	1.15 (0.52–2.55)
Vomiting	1.31 (0.31–5.53)	1.29 (0.22–7.63)
Headache	0.97 (0.60–1.57)	0.99 (0.59–1.67)
Diarrhea	0.59 (0.10–3.60)	1.30 (0.24–6.96)
Earache (dummy symptom)	2.45 (0.70–8.64)	2.77 (0.73–10.57)
Q16 symptoms		
Painful tingling in some parts of the body*,***	3.05 (1.06–8.81)	3.36 (1.13–9.98)
Less interested in sex than what you think is normal**	2.79 (0.82–9.46)	3.62 (1.07–12.25)
Irritated without any particular reasons*	2.23 (1.08–4.60)	1.84 (0.85–3.98)
Relatives told that you have short memory	1.74 (0.92–3.31)	1.65 (0.84–3.23)
Problems with concentrating	1.58 (0.91–2.73)	1.22 (0.67–2.23)
Often go back and check things**	1.53 (0.85–2.74)	2.04 (1.11–3.72)
Depressed without any particular reasons	1.51 (0.76–2.98)	1.38 (0.67–2.83)
Difficulty breathing[b]	1.49 (0.70–3.19)	1.79 (0.81–3.95)
Short memory	1.34 (0.80–2.24)	1.39 (0.81–2.40)
Abnormally tired	1.31 (0.81–2.10)	1.30 (0.78–2.16)
Palpitations even without exertion	1.24 (0.63–2.44)	1.35 (0.65–2.79)
Oppression of the chest	1.16 (0.64–2.11)	1.54 (0.82–2.87)
Headache at least once a week	1.12 (0.70–1.79)	1.17 (0.70–1.96)
Trouble falling asleep[b]	1.10 (0.68–1.78)	0.93 (0.55–1.58)
Perspiration without any particular reason***	1.08 (0.64–1.83)	0.90 (0.50–1.62)
Constipation (dummy symptom)[b]	1.66 (0.62–4.50)	0.71 (0.23–2.13)

[a]Adjusted for age and years of education.
[b]Question different from Q16 of Hogstedt et al. [1984].
*$P < 0.05$, Cox's regression, general farmers versus controls.
**$P < 0.05$, Cox's regression, IPM-farmers versus controls.
***$P < 0.05$, Cox's regression, >13% inhibition.

cholinesterase inhibition and Q16 symptoms related to exposure variables. The crude prevalence ratios differed only slightly from the adjusted prevalence ratios. Farmers had increased prevalence ratios for acute, inhibition related, as well as Q16 symptoms, compared with controls. Cholinesterase inhibition (more than 8% (median) and more than 13% (25 percentile)) was significantly associated with increased prevalence of dichotomized acute and inhibition related symptoms among the entire group of farmers and controls. Cholinesterase inhibition above 13% was also significantly associated with increased acute and inhibition related symptom prevalence among farmers only. Previous organophosphate poisoning and having sprayed insecticides during *Maha* season were positively though not significantly

TABLE VI. Factors Possibly Associated With 16 Acute Organophosphate or Carbamate Exposure Related Symptoms (>6/16), Seven Acute Symptoms Related to Acetylcholinesterase Inhibition (>3/7), and Q16 Symptoms (>6/16) Among Sri Lankan Farmers and Controls

	Adjusted prevalence ratios (95% CI)[a]		
Factor	**Symptoms experienced during last week**	**Symptoms related to AChE inhibition**	**Q16 symptoms**
Farmers and controls (n = 260)			
Farmers versus controls	2.85 (1.21–6.74)*	5.04 (1.55–16.38)*	1.59 (0.93–2.70)
Cholinesterase inhibition ≥8% (median)	1.60 (1.03–2.47)*	1.65 (1.02–2.66)*	1.06 (0.76–1.46)
Cholinesterase inhibition ≥13% (25 percentile)	2.02 (1.32–3.08)*	2.54 (1.60–4.02)*	1.22 (0.86–1.73)
Farmers only (n = 216)			
Did not receive IPM training	1.24 (0.75–2.03)	1.31 (0.77–2.23)	1.04 (0.70–1.54)
Cholinesterase inhibition ≥8% (median)	1.41 (0.89–2.23)	1.41 (0.86–2.31)	1.04 (0.73–1.48)
Cholinesterase inhibition ≥13% (25 percentile)	1.79 (1.15–2.79)*	2.14 (1.33–3.44)*	1.15 (0.79–1.66)
Suffered from acute pesticide poisoning in the past (OP or carbamate poisoning symptoms)	1.54 (0.92–2.55)	1.42 (0.83–2.42)	1.16 (0.75–1.79)
Received medical treatment for acute pesticide poisoning in the past	1.49 (0.87–2.55)	1.42 (0.80–2.55)	0.95 (0.58–1.54)
Sprayed insecticides more than 13 hr per hectare during *Maha* season (median)	1.18 (0.74–1.88)	1.16 (0.70–1.90)	1.11 (0.77–1.61)
Sprayed insecticides more than 38 hr per hectare during *Maha* season (75 percentile)	1.22 (0.75–1.98)	1.15 (0.68–1.95)	1.07 (0.72–1.59)

[a]Adjusted for age and years of education.
*$P < 0.05$, Cox's regression.

associated with increased prevalence of the dichotomized symptoms.

DISCUSSION

This study showed that a group of Sri Lankan farmers had significantly higher red blood cell cholinesterase inhibition during a high pesticide exposure period than a control group of fishermen. The higher inhibition among farmers was obviously due to occupational insecticide exposure since no other environmental exposures are known that lower erythrocyte cholinesterase [Ciesielski et al., 1994]. Farmers who had followed an IPM-demonstration had less cholinesterase inhibition than farmers not trained in IPM, probably because they sprayed significantly fewer insecticides. Farming was also associated with higher prevalence of both acute and chronic symptoms related to pesticide exposure. The acute symptoms were positively and significantly associated with acetylcholinesterase inhibition (more than 13% inhibition; $P < 0.05$). Dichotomized acute symptoms and acute symptoms associated with cholinesterase inhibition showed a dose–response relationship for cholinesterase inhibition and symptom prevalence. Although Q16 symptoms were more prevalent in farmers than in controls, no relationships existed between Q16 prevalence and inhibition, spraying insecticides in *Maha* season or IPM-farming, probably because these factors do not reflect chronic exposure to insecticides.

Several forms of bias could have affected the outcome of the study. However, it is unlikely that selection bias influenced the results since all invited farmers and controls agreed to participate. In addition, the initial and final study population was comparable regarding personal characteristics, symptoms and baseline cholinesterase level. Responder bias could have played a role because objectives of the study were explained to all participants. Farmers might have over-reported their health complaints because they were aware of the problem of pesticide exposure. However, dummy symptoms showed similar prevalence for both groups, thus it is unlikely that the relationships between farming and symptom prevalence is due to responder bias.

Poor recall of spray activities during the previous month (especially pesticides' names) affected associations between spraying, and cholinesterase inhibition or symptoms. As it was essential to know whether organophosphates or carbamates were involved it was not possible to use this information as a marker of exposure. It was expected that farmers who frequently sprayed pesticides during the entire preceding *Maha* season would also be more high by exposed during *Yala*. However, a dose–response relationship for spraying insecticides during the preceding *Maha* season and acetylcholinesterase inhibition was not seen, probably explaining the absence of a clear relationship between spraying and symptom prevalence. Acetylcholinesterase inhibition was, therefore, a better proxy for recent exposure to cholinesterase-inhibiting insecticides than self-reported

spray-activities during either *Maha* season or the previous month. It is unlikely that differences found between general farmers and IPM-farmers with regard to insecticide use during *Maha* season are due to information bias because reported use of other pesticides was similar.

As other factors may also be related to the symptom-questionnaires it was important to control for potentially confounding factors such as age, sex, alcohol use, smoking, educational level, previous organophosphate poisoning, and nutritional status. The prevalence of most symptoms was higher among both farmers and controls, probably due to a low level of standard of living. Although age did not meaningfully affect the results after correction, it was decided to keep it in the regression model. Alcohol intake and tobacco consumption was high for all study subjects, however adjustment for alcohol and tobacco did not meaningfully change associations with symptoms. Subjects who had suffered from organophosphate poisoning showed a (non-significantly) elevated symptom prevalence, possibly as a sequel of acute poisoning. However, it was decided not to correct for previous poisoning as it did not meaningfully change symptom prevalence ratios. Lower educational level (total years) was the only potential confounding factor that was significantly related to increasing symptom prevalence.

Cholinesterase inhibition among farmers was lower than in a study conducted in Kenya where an average inhibition level of 35% was found among exposed farm workers [Ohayo-Mitoko et al., 2000]. This difference might be due to different timing of taking blood samples; in the Kenyan study blood samples of pesticide applicators of both small and large-scale farms were obtained at the end of work shifts whereas data collection in Sri Lanka took place per village. Also, the Sri Lankan farmers were used to spraying on an irregular basis and not all farmers had recently been exposed to organophosphates or carbamates at the time of blood sampling, which could explain the relatively low average inhibition of 9%. Surprisingly the control group had a lower average cholinesterase activity than both groups of farmers during the low exposure period. Possibly the production of cholinesterase among farmers is increased because of their repeated exposure to cholinesterase-inhibiting pesticides; human beings may develop a compensation mechanism and therefore, farmers may have higher cholinesterase levels than controls in low exposure periods [Ciesielski et al., 1994]. The WHO recommends 30% cholinesterase inhibition as the level for removal of workers from exposure [IPCS, 1986]. The Kenyan study suggested that increased respiratory, eye, and central nervous system symptom prevalence might occur at cholinesterase inhibition levels below this level, which is in agreement with the findings of this study.

The group of farmers that had attended IPM demonstrations reported considerably less insecticide use than general farmers. Promotion and implementation of intensive, participatory IPM training (e.g., the Farmer Field School concept) may be a sustainable approach to reduce insecticide use even more. Although IPM-farmers experienced lower cholinesterase inhibition than general farmers, and somewhat lower acute symptoms prevalence, insecticide exposure still seemed to result in depressed cholinesterase levels and increased symptom prevalence.

Protective clothing and determinants of hygienic behavior were not related to the level of ACh-inhibitions. However, our study design was not appropriate for the evaluation of protective measures and therefore, no conclusion can be drawn from these results. Intervention studies should be performed to evaluate the effect of control measures.

The Mahaweli Authority of Sri Lanka promotes crop diversification from paddy, towards banana and vegetables as a water saving strategy in Uda Walawe. According to this study, insecticide use per hectare is much higher in vegetables than in paddy and banana so the promotion of crop diversification towards vegetables could have a negative impact on the health of farmers in Uda Walawe while diversification towards banana could have a positive impact.

Our results suggest that occupational acetylcholinesterase-inhibiting insecticide exposures have a negative impact on the health of farmers in Uda Walawe. A high proportion (24%) of farmers suffered at least once from acute occupational pesticide poisoning in the past. Insecticide use seemed to result in depressed cholinesterase levels and cholinesterase inhibition was associated with increased acute symptom prevalence. IPM training seemed to result in less insecticide use, and less cholinesterase inhibition (8 vs. 11%; $P < 0.01$). However, only a small effect of IPM training on the prevalence of acute pesticide-related symptoms could be determined and no effect was found for chronic neurological symptom prevalence.

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APPENDIX

Pesticides Used Last Month During Baseline and *Yala* Season by General Farmers (no IPM, 275 Spray Operations) and IPM-Farmers (IPM, 239 Spray Operations) in Sri Lanka

Chemical type and common name of pesticide	WHO classification[a]	No IPM, percentage of all spray operations	IPM, percentage of all spray operations
Insecticides		71.3	53.7
OP/carbamate insecticides		40.4	34.4
Herbicides		23.6	38.0
Fungicides		5.1	8.3

APPENDIX (*Continued*)

Chemical type and common name of pesticide	WHO classification[a]	No IPM, percentage of all spray operations	IPM, percentage of all spray operations
Organophosphate insecticides			
Chlorpyrifos	II	10.2	12.6
Fenthion	II	4.4	0.8
Dimethoate	II	4.0	5.9
Phenthoate	II	2.2	0.4
Profenophos	II	1.8	2.5
Quinalphos	II	1.5	1.7
Prothiophos	II	0.7	0.0
Methamidophos	Ib	0.7	1.3
Oxydemeton-methyl	Ib	0.7	0.0
Monocrotophos	Ib	0.4	0.0
Edifenphos (fungicide)	Ib	0.0	1.7
Diazinon	II	0.0	0.4
Carbamate insecticides			
Carbosulfan	II	6.5	0.4
Methomyl	Ib	2.9	1.3
Fenobucarb (BPMC)	II	1.8	2.1
Carbofuran	Ib	1.5	2.9
Carbaryl	II	0.7	0.0
Thiodicarb	II	0.4	0.4
Organochlorine insecticides			
Endosulfan	II	3.6	0.8
Pyrethroid insecticides			
Permethrin	II	4.4	0.8
Deltamethrin	II	3.3	0.0
Ethofenprox	0	2.5	0.0
Fenvalerate	II	0.4	1.3
Other insecticides		16.7	16.3
Herbicides			
Glyphosate	0	8.0	2.5
Propanil	III	6.5	12.6
Paraquat	II	4.0	3.3
MCPA	III	2.9	9.6
Other herbicides		2.2	10.0
Fungicides			
Thiophanate-methyl	0	1.8	0.8
Sulfur	0	1.1	1.3
Captan	0	0.7	0.4
Benomyl	0	0.4	0.8
Other fungicides		1.1	5.0

Pesticides were mostly applied by backpack spraying.
Pesticides are classified as Class Ia (extremely hazardous), Class Ib (highly hazardous), Class II (moderately hazardous), Class III (slightly hazardous), Table V (0) (unlikely to present acute hazard in normal use) [WHO, 2001].
[a]The WHO bases its acute hazard classification on the lowest published rat oral LD50, the lethal dose (in milligrams of substance per kilogram of body weight) that kills 50% of the test animals in a standard assay.