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Original Research Article

Blood cholinesterase level and its association with neurobehavioral performance due to insecticide exposure among male cocoa farmers in Pahang and Perak, Malaysia

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Abstract

Background: Chlorpyrifos (group II) is an example of organophosphate (OP) insecticides used in cocoa farm that contains moderately hazardous substances that may be dangerous, toxic and can cause severe effects to human health. It is a concern considering cocoa plantations uses large amount of pesticides. There is a lack of study focusing on cocoa farmers' exposure to organophosphate and data is needed to allow preventive measures to be planned and implemented in order to protect the health and safety of the workers in this specific agricultural sector.

Objective: This study aims to determine blood cholinesterase level due to exposure to organophosphate pesticides and identify its link with neurobehavioral performance of cocoa farmers in cocoa plantations across Perak and Pahang, Malaysia.

Method: This is a cross-sectional study among participants that includes cocoa farmers as the exposed group and fishermen as the comparison group. Structured questionnaire were administered to obtain background information and occupational usage of insecticides. Blood samples were collected via finger prick technique and seven tests of World Health Organization (WHO) Neurobehavioral Core Test Battery (NCTB) were used to measure the neurobehavioral performance of the respondents. Data collected were entered into statistical software and were analysed according to objectives.

Results: A total of 178 respondents were recruited in this study. This study had reversed results where the blood cholinesterase level of the comparison group was below than the normal range compared to the exposed group. The standard scores of Pursuit Aiming and Trail Making tests showed that the exposed group had significantly poorer performance than the comparison group. There was no significant correlation between the blood cholinesterase with neurobehavioral performance scores for the exposed group. Income, years of handling were significant predictor factors for Pursuit Aiming Test whereas age, use of gloves and education for Trail Making Test.

Conclusion: The findings of this study showed that neurobehavioral assessment conducted have provided insight into the early effects of OP pesticide exposures when results detected impairment of cocoa farmers' motor steadiness and visual motor coordination. There is a need to encourage commitment of employers to protect workers through conducting medical surveillance, reviewing existing training modules, assist in the amendment of the existing guideline for use and provide adequate and suitable personal protective equipment for the use of the workers in order to fulfil the objectives of the Occupational Safety and Health Act.

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Keywords: Organophosphate**,** blood cholinesterase level, neurobehavioral performance, cocoa farmers

Introduction

Malaysia is one of the countries in Asia that produces cocoa for the manufacturing of cocoa-based products such as cocoa butter, cocoa paste, cocoa powder and chocolate. The total size of cocoa plantation in Malaysia is approximately 29,951 hectare in 2004 (Malaysia Cocoa Board, 2004) where majority of cocoa is cultivated in Sabah and Sarawak. However, in Peninsular Malaysia, cocoa farms are more concentrated in Pahang, Perak and Kelantan. Organophosphate (OP) insecticides that has been widely used in local cocoa farms contains hazardous substances that may be dangerous, toxic and can cause severe effects such as acute affect (eye irritation, skin irritation) and chronic effect (birth defect, neurodevelopment problems) to the human health. It has been established by the Insecticide Resistance Action Committee (IRAC) that OP insecticide had been classified as Groups 1A Cholinesterase Inhibitor which will increase cholinergic effects of neurotransmitter, acetylcholine in body and depolarize neural transmission, resulting in damage to neurological functioning (Weiss et al. 2004). Evidence have shown associations between OP exposure and neurobehavioral impairment in adult populations of farmers and farmworkers (Baldi et al. 2011; Kamel et al. 2003).

The Malaysian Cocoa Board have reported that farms use approximately 10-15 litres of pesticides for one hectare of plantation in a three months' cycle of cocoa planting in the Peninsular Malaysia (Malaysia Cocoa Board, 2016). However, occupational exposures and published data regarding pesticide exposure among cocoa farmers is lacking in literature. Considering that pesticides including OP pesticides are used in plantation fields, the biggest concern would be the exposure experienced by cocoa farm workers if there were no proper emphasis for occupational health and safety. The legislation requirement as specified in the Occupational Safety and Health Act 514 1994 (Use and Standard of Exposure of Chemicals Hazardous to Health) Regulation 2000 in Malaysia have made it mandatory that workers who are exposed to OP to undergo routine medical surveillance of cholinesterase activity in red cells and plasma. Although trainings such as Standard of Practice (SOP) for spraying pesticides and personal protective equipment (PPE) use have been reported to be provided, the effectiveness of these control methods have not been reported in literature. Furthermore, there is a need to assess the health effects among cocoa farmers from the usage of those pesticides to enhance the importance of the trainings provided. Currently, in Malaysia there are limited studies which has been done to identify the effects of OP on blood cholinesterase level and neurobehavioral performance specifically among cocoa farmers unlike other agricultural sectors as reported in literature (Lokhman et al., 2015; How et al., 2014). As such, this study aims to determine the blood cholinesterase level and its association with neurobehavioral performance of cocoa farmers in cocoa plantations across Perak and Pahang, Malaysia due to exposure to OP pesticides. This study will hopefully provide basic information on pesticide exposures among this sub group of agricultural workers.

Materials and Method

This study employs a cross-sectional design to determine blood cholinesterase levels and neurobehavioral performance and its link with occupational OP exposures among cocoa farmers in Sungai Sumun, Perak and Jengka, Pahang. The locations were selected because these are the only two plantations and research centre for cocoa in Peninsular Malaysia. Blood cholinesterase levels in this study act as the biomarker of exposure to OP. Before the study was conducted, institutional ethical approval was sought. This study had received approval from the Universiti Putra Malaysia Ethics Committee Board prior to data collection. The Malaysian Cocoa Board granted the approval for the study to be conducted.

The sampling frame were cocoa farmers who have been working in Cocoa Plantation and Research Centre at Pahang and Perak and those who handles pesticides in the workplace. A list of 96 cocoa farmers in Peninsular Malaysia was obtained from the Malaysian Cocoa Board. Purposive sampling was used to select samples as only certain cocoa farmers with inclusive and exclusive criteria that could provide desired information related to OP use were invited to take part in this study. The inclusion and exclusion criteria used in this study is as presented in Table 1 and Table 2 respectively. This study uses several types of methodology, namely questionnaire, cholinesterase test kit and seven tests of neurobehavioral performance instruments.

Table 1: Inclusion and Exclusion Criteria of the Study – Exposed Group

Inclusion criteria	Exclusion criteria
• Male adults • 20 – 60 years old • Have at least two years of farming experience. • Particularly involved in handling of OP pesticides. • No diagnosis of chronic disease such as cancer, kidney failure, liver failure, chemotherapy. • Not mentally and physically affected such as not mentally retarded, able to see, read, write, and speak and response well.	• Work as part time pesticides handler. • Work outside other than in cocoa farms. • Have history of head injury. • Has alcohol intake.

Table 2: Inclusion and Exclusion criteria of the study – Comparison Group

Inclusion criteria	Exclusion criteria
• Male adults. • 20 – 60 years old. • No direct contact or history with handling of pesticides specifically OP in farm. • No diagnosis of chronic disease such as cancer, kidney failure, liver failure, chemotherapy. • Able to see, read, write, and speak and response well. • Malaysian	• Not able to read, write, speak and response well. • Reported alcohol intake.

Study instruments

Questionnaire

The questionnaire used in this study was adapted from several studies (Holtman, 2013; Al-Haddad and Al Sayyad, 2013; Lorenz, 2011; Nordin et al. 2002; Yassin et al. 2002; World Health Organization, 1986) and was modified according to the setting of this study. The interview-based questionnaire was provided in Malay. There were two different sets of questionnaires; namely for the exposed and the comparison group. The questionnaire for exposed group (cocoa farmers) consisted of eight parts namely Part A: Demographic Information, Part B: Background information, Part C: Background Information on Working History, Part D: Information on Use of Pesticides at Workplace, Part E: Information on Use of Personal Protective Equipment (PPE), Part F: Work Practices when Handling Pesticides, Part G: Other Information and Part H: Health Information. On the other hand, the questionnaire for the comparison group only consisted of Part A, B, C and H.

Pre-test of questionnaires were conducted prior to data collection among representatives of cocoa farmers, to observe whether the questionnaire can be understood and to avoid any unfamiliar terms. The questionnaire were also tested for its reliability.

Cholinesterase Test Kit

In this study, a competent person conducted all blood collection from all subjects. About 10 µl of blood from each respondent was obtained using finger prick technique. Blood collection was only taken once for baseline and only to observe chronic exposure. Then the blood samples were analysed using LOVIBOND AF267 Cholinesterase Test Kit. All containers containing biological specimen were labelled with only identification code to ensure confidentiality.

Specimen collected were stored in refrigerator or freezer and were allowed to stand for at least 20 to 30 minutes to reach room temperature before analysis. The kits and specimens were stored in room temperature (not exceeding 30ºC). The expiration date of the test kit and chemicals were ensured before use.

The substrate solutions were freshly prepared daily and any samples or solutions that had air bubbles were eliminated. Blood cholinesterase levels were categorised according to the specific range given by the manufacturer. The three categories were normal (100-75%), overexposure (74.9-50%) and serious overexposure (49.9-25%).

Neurobehavioral Core Test Battery (NCTB)

The neurobehavioral Core Test Battery (NCTB) assessment was used to determine the health effects measured in this study. The full set of test took about 30-45 minutes to complete and its results were compared between the two groups. NCTB includes the following subtests: Benton Visual Retention, Digit Symbol, Digit Span, Pursuit Aiming, Trail Making, Santa Ana Manual Dexterity and Simple Reaction Time. Each subtest was administered to all subjects by only one specific researcher. All testing procedures were understood and practiced by the researchers to avoid any bias. Furthermore, testing was conducted in an area where it was free from distracting noise, had adequate lighting and comfortable room temperature. All instructions given to respondents were loud, concise and clear. In terms of scoring, the standard score below 50 indicates that a respondent had poor neurobehavioral performance whereas standard score above 50 indicates good neurobehavioral performance level (WHO, 1986).

Statistical Analysis

All analysis was performed using the IBM SPSS (Statistical Package for Social Science), version 22. The significance level was set at $p<0.05$. The χ^2 and Fisher's Exact tests were performed to compare socio demographic characteristics, blood distribution and neurobehavioral scores while Mann-Whitney U test was performed to identify significant differences for blood cholinesterase concentration. Pearson and Spearmen correlations were used to evaluate associations between organophosphate exposures with neurobehavioral performance level. To identify significant predictors of the selected neurobehavioral performance, multiple linear regression was performed.

Result

Background Information of the Respondents

Data collection was conducted between January to May 2017. The number of respondents who participated in this study was 178 people. For the exposed group, a total of 90 from 96 cocoa farmers were recruited while for the comparison group, 88 out of 94 fishermen agreed to participate in this study. The response rate for this study was 93%.

All respondents were males and were of Malay ethnicity. Three-quarter of the exposed group were of younger age group (less than 40 years of age), more than 90% of them had secondary level of education, half of them had income of more than RM3000 and above, almost 80% of them had an occupational tenure of less than 15 years. There were higher percentage of the comparison group who were living within 5 km of agricultural area when compared to exposed group. For both groups of respondents, more than 70% of them smokes and about one-third of them had normal BMI. Table 3 presents the distribution of socio-demographic characteristics of the respondents.

Blood Cholinesterase Level

The average (mean and standard deviation, SD) for blood cholinesterase concentration (in percent) for the exposed group was 86.7 (13.5) % and for comparison group was 79 (17.7) %. Lower level of cholinesterase concentration was observed for the exposed group (cocoa farmers). Table 4 presents the blood cholinesterase levels of the two groups of respondents in this study.

Distribution of Blood Cholinesterase Concentration

Blood cholinesterase concentration of the respondents were categorised into three groups. It was noted that this study had reversed results whereby there were higher percentage of the comparison group in the overexposure and serious overexposure category (21.6%) when compared to exposed group (8.9%). The distribution of blood cholinesterase is as presented in Table 5.

Neurobehavioral Performance

For the neurobehavioral performance, only Pursuit Aiming (χ^2: 39.66; $p < 0.05$) and Trail Making test (χ^2:7.676; $p < 0.05$) showed a significant difference between the exposed and comparison group. Higher percentage of respondents in the exposed group (98.9% for Pursuit Aiming, 51.1% for Trail Making) performed poorer in both tests compared to the comparison group (61.4% for Pursuit Aiming, 30.7% for Trail Making) that scored below the score of 50. Table 6 presents the cross-tabulation of the distribution of neurobehavioral test scores between the two groups.

The Relationship between Blood Cholinesterase Level and Neurobehavioral Performance

Table 7 shows the correlation between blood cholinesterase levels with NCTB test scores. There was no significant correlation between blood cholinesterase levels with all neurobehavioral performance scores for the exposed group.

Factors Influencing Neurobehavioral Impairment

Table 8 showed the selected variables included in the regression analysis for Pursuit Aiming test. Simple linear regression was carried out prior to selecting variables to be included in further analysis. Variables that were included had p-value of less than 0.25 in the Simple Linear Regression or are determined to be clinically important. From the overall selected variables, income and years of handling organophosphate pesticides were the significant predictor factors ($p<0.05$) for Pursuit Aiming test of the exposed group. In this study, the variables explained 14.1% of the outcome.

Table 9 presents the regression analysis for Trail Making test. Variables such as age, education level and use of gloves were the significant predictor factors ($p<0.05$) for Trail Making test. In this test, 25.7% of the variables explained the outcome of the Trail Making test. An increase of age were inversely linked with the standard score of the Trail Making test while and an increase of education level and the use of gloves were linked were linked to increased score of the Trail Making test.

Table 3: Cross-tabulation of socio-demographic characteristics of the exposed (farmers) and comparison (fishermen) groups

Variables	Exposed N (%) (n=90)	Comparison N (%) (n=88)	χ^2	*p-value*
Age (years)				
20 – 29	27 (30)	5 (5.7)		
30 – 39	34 (37.8)	12 (13.6)		
40 – 49	15 (16.7)	29 (33)	44.1[a]	<0.05*
50 – 60	14 (15.6)	42 (47.7)		
BMI				
Underweight	5 (5.6)	4 (4.5)		
Normal	39 (43.3)	30 (34.1)		
Overweight	29 (32.2)	36 (40.9)	2.09[b]	0.55
Obesity	17 (18.9)	18 (20.5)		
Education Level				
Primary school	5 (5.6)	23 (26.1)		
Secondary school	78 (86.7)	60 (68.2)	14.2[a]	<0.05*
STPM/Diploma	7 (7.8)	5 (5.7)		
Income (RM)				
<1500 (USD381.60)	47 (52.2)	68 (77.3)		
1501(USD381.85) – 3000 (USD763.20)	38 (42.2)	18 (20.5)	12.2[b]	<0.05*
>3000 (USD763.20)	5 (5.6)	2 (2.3)		
Working Years (years)				
<15	70 (77.8)	42 (47.7)		
16 – 30	18 (20)	30 (34.1)		
>30	2 (2.2)	16 (18.2)	20.9[a]	<0.05*
Distance from home to any agricultural area				
Less than 5 kilometres	48 (53.3)	80 (90.9)		
5-10 kilometres	31 (34.4)	6 (6.8)	31.1[a]	<0.05*
More than 10 kilometres	11 (12.2)	2 (2.3)		

Smoking Status				
Smoker	64 (71.1)	68 (77.2)		
Non-smoker	26 (28.9)	20 (22.7)	0.88[a]	0.35
Number of Cigarettes (per day) (n=64)				
1 – 20	58 (90.6)	61 (89.7)		
21 – 40	4 (6.3)	7 (10.3)	2.39[b]	0.28
41 - 60	2 (3.1)	0 (0)		
Years of smoking (years) (n=64)				
<15	29 (45.3)	6 (8.8)		
16 – 35	29 (45.3)	53 (77.9)	22.6[a]	<0.05*
>35	6 (9.4)	9 (13.2)		

N=178 **p*-value significant at 0.05 level
[a] Chi-square tests [b] Fisher's Exact Test with Freeman-Halton extension

Table 4: Blood Cholinesterase Concentrations of the Respondents

Variable	Median (IQR)		Mean (SD)		z	*p-value*
	Exposed	Comparison	Exposed	Comparison		
Blood Cholinesterase Level (%)	87.5 (25)	75 (25)	86.7 (13.5)	79 (17.7)	-2.889	0.004*

N=178
**p*-value significant at 0.05 level
Blood cholinesterase level nearing value of 100 indicates normal blood cholinesterase level.
IQR= Interquartile range SD = Standard Deviation
Mann-Whitney U Test

Table 5: Distributions and Comparison of Blood Cholinesterase Concentration

Group	Frequency (%)			$\chi 2$	*p-value*
	Normal	**Overexposure**	**Serious Overexposure**		
Exposed	82 (91.1)	7 (7.8)	1 (1.1)	5.64	0.04*
Comparison	69 (78.4)	17 (19.3)	2 (2.3)		
Both Groups	151 (84.8)	24 (13.5)	3 (1.7)		

N=178
**p*-value significant at 0.05 level
Fisher's Exact Test with Freeman-Halton extension

Table 6: Comparison of Category of Neurobehavioral Performance Level between Two Groups

Benton Visual Retention Test				
	Below 50 (%)	**Above 50 (%)**	χ^2	*p-value*
Group			1.804	0.179
Exposed	33 (36.7)	57 (63.3)		
Comparison	41 (46.6)	47 (53.4)		
Digit Span Test				
	Below 50 (%)	**Above 50 (%)**	χ^2	*p-value*
Group			1.900	0.168
Exposed	46 (51.1)	44 (48.9)		
Comparison	54 (61.4)	34 (38.6)		

Digit Symbol Test				
	Below 50 (%)	Above 50 (%)	χ^2	*p-value*
Group			0.026	0.871
Exposed	48 (53.3)	42 (46.7)		
Comparison	48 (54.5)	40 (45.5)		
Time Reaction Test				
	Below 50 (%)	**Above 50 (%)**	χ^2	***p-value***
Group			2.706	0.100
Exposed	52 (57.8)	38 (42.2)		
Comparison	40 (45.5)	48 (54.5)		
Pursuit Aiming Test				
	Below 50 (%)	**Above 50 (%)**	χ^2	***p-value***
Group			39.66	<0.05*
Exposed	89 (98.9)	1 (1.1)		
Comparison	54 (61.4)	34 (38.6)		
Trail Making Test				
	Below 50 (%)	**Above 50 (%)**	χ^2	***p-value***
Group			7.676	<0.05*
Exposed	46 (51.1)	44 (48.9)		
Comparison	27 (30.7)	61 (69.3)		
Santa Ana Dominant Test				
	Below 50 (%)	**Above 50 (%)**	χ^2	***p-value***
Group			1.092	0.296
Exposed	40 (44.4)	50 (55.6)		
Comparison	46 (52.3)	42 (47.7)		
Santa Ana Non-Dominant Test				
	Below 50 (%)	**Above 50 (%)**	χ^2	***p-value***
Group			0.028	0.868
Exposed	49 (54.4)	41 (45.6)		
Comparison	49 (55.7)	39 (44.3)		

N=178
**p*-value significant at 0.05 level
Greater neurobehavioral performance denoted by score above 50, poor neurobehavioral performance denoted by score below 50.
Chi-Square Test

Table 7: The Relationship between Blood Cholinesterase Level and Neurobehavioral Performance

Variables	Blood Cholinesterase Level (%)			
	Exposed		Comparison	
	r	*p-value*	*r*	*p-value*
Benton Visual Retention Test[b]	0.111	0.297	-0.124	0.251
Digit Span Test[b]	-0.011	0.916	-0.251	0.018*
Digit Symbol Test[a]	0.160	0.131	-0.201	0.060
Time Reaction Test[b]	0.065	0.541	-0.105	0.330
Pursuit Aiming Test[b]	-0.200	0.059	0.018	0.866
Trail Making Test[b]	-0.006	0.954	-0.194	0.071
Santa Ana Dominant Test[a]	0.125	0.240	-0.142	0.187
Santa Ana Non-Dominant Test[a]	0.175	0.098	-0.282	0.008**

N=178
**p*-value significant at 0.05 level
***p*-value significant at 0.01 level
[a] Pearson Correlation
[b] Spearman Correlation

Table 8: Regression analysis for selected variables for Pursuit Aiming Test

Variables	SLR[a]		MLR[b]				
	B (95% CI)	*p-value*	Adjusted B	t-statistics	*p-value*	F	*p-value*
(Constant)			50.032	34.224	<0.05*	2.351	0.017*
Blood Cholinesterase Level	-0.005 (-0.028, 0.017)	0.64	0.001	0.072	0.94		
BMI			0.275	1.572	0.12		
Underweight	-0.176 (-1.507, 1.154)	0.79					
Normal	-0.249 (-0.862, 0.364)	0.42					
Overweight	-0.093 (-0.746, 0.559)	0.78					
Obesity	0.592 (-0.177, 1.361)	0.13					
Income (RM)			0.967	3.203	<0.05*		
<1500 (USD381.60)	-0.126 (-0.736, 0.484)	0.68					
1501 (USD381.85) – 3000 (USD763.20)	-0.471 (-1.080, 0.139)	0.13					
>3000 (USD763.20)	2.788 (1.595, 3.981)	<0.05*					
Smoking Status			-0.080	-0.159	0.87		
No	0.577 (-0.085, 1.239)	0.09					
Yes	-0.577 (-1.239, 0.085)	0.09					
Years of Smoking (years)	-0.020 (-0.045, 0.005)	0.12	-0.014	-0.663	0.51		
Years of Living in Current Home (years)	-0.017 (-0.040, 0.007)	0.16	-0.011	-0.990	0.33		
Years of Handling Pesticides (years)	-0.042 (-0.088, 0.004)	0.07	-0.086	-2.912	<0.05*		
Use of PPE			-0.384	-0.592	0.56		
No	-0.176 (-1.507, 1.154)	0.79					
Yes	0.176 (-1.154, 1.507)	0.79					
Taking bath after handling pesticides			-0.184	-0.317	0.75		
No	-0.320 (-1.390, 0.749)	0.55					
Yes	0.320 (-0.749, 1.390)	0.55					
Time of change to new clothes after handling pesticides			-0.242	-0.889	0.38		
Immediately	0.500 (-0.103, 1.103)	0.10					
Before lunch time	-0.415 (-1.019, 0.189)	0.18					
At the end of work	-0.388 (-1.717, 0.941)	0.56					

N=90 **p*-value significant at 0.05 level [a] Simple Linear Regression [b] Multiple Linear Regression
Income and Years of Handling Pesticides were significantly linked with Pursuit Aiming Test score of exposed group

Table 9: Regression analysis for selected variables for Trail Making Test

Variables	SLR[a]		MLR[b]				
	B (95% CI)	*p-value*	Adjusted B	t-statistics	*p-value*	F	*p-value*
(Constant)			52.555	7.881	<0.05*	2.739	0.006*
Blood Cholinesterase Level	-0.015 (-0.141, 0.110)	0.81	0.007	0.112	0.91		
Age	-0.233 (-0.389, -0.077)	<0.05*	-0.205	-2.134	<0.05*		
Education Level			4.675	2.133	<0.05*		
Primary School	-2.000 (-9.341, 5.341)	0.59					
Secondary School	-2.628 (-7.552, 2.295)	0.29					
STPM/Diploma	5.697 (-0.475, 11.869)	0.07					
Income (RM)			-0.368	-0.224	0.82		
<1500 (USD381.60)	2.771 (-0.549, 6.091)	0.10					
1501(USD381.85)–3000 (USD763.20)	-2.677 (-6.040, 0.685)	0.12					
>3000 (USD763.20)	-0.729 (-8.081, 6.622)	0.84					
Smoking Status			2.528	1.349	0.18		
No	-2.440 (-1.318, 0.191)	0.19					
Yes	2.440 (1.318, 0.191)	0.19					
Tenure (years)	-0.219 (-0.451, 0.014)	0.07	0.228	0.896	0.37		
Years of Handling Pesticides (years)	-0.260 (-0.515, -0.006)	<0.05*	-0.223	-0.832	0.41		
Use of Gloves			3.768	2.334	<0.05*		
No	-3.889 (-7.227, -0.551)	<0.05*					
Yes	3.889 (0.551, 7.227)	<0.05*					
Location of washing hands after handling pesticides			-0.391	-0.767	0.45		
Near the farm area with bottled water	0.069 (-4.141, 4.280)	0.97					
Toilet located at pesticide storage	3.757 (-0.081, 7.594)	0.06					
Others	-3.822 (-7.092, -0.552)	<0.05*					
Location of taking bath after handling pesticides			-2.170	-1.911	0.06		
Toilet located at pesticide storeroom	3.580 (-1.983, 9.143)	0.20					
Toilet at home	2.533 (-0.999, 6.066)	0.16					
Others	-5.894 (-10.604, -1.364)	<0.05*					
Not applicable	-1.799 (-7.705, 4.107)	0.55					

N=90 **p*-value significant at 0.05 level [a] Simple Linear Regression [b] Multiple Linear Regression
Age, Education Level and Use of Gloves were significantly linked with Trail Making Test score of exposed group

Discussion

Blood Cholinesterase Level

This study was able to assess the cholinesterase levels and measure neurobehavioral performance tests of 178 respondents from the population of cocoa farmers and fishermen in Perak and Pahang. In general, this study showed contradictory results by which the comparison group had lower blood cholinesterase level than the exposed group. It was found that all the comparison respondents in Perak area had blood cholinesterase level in normal range but results from the Pahang area was contrary to the hypothesis of this study, where the blood cholinesterase level of those who were not occupationally exposed to organophosphate was below than the normal range for most of them.

To explain this contrary results, several factors were considered. For the inhibition of blood cholinesterase, there are other chemicals that could cause similar neurotoxicity mechanism like organophosphate such as polycyclic aromatic hydrocarbons, detergents, heavy metals and contaminant mixtures (Bressler and Goldstein, 1991). There could be possibility of the presence of other chemicals that were not expected such as heavy metals (Abdullah, 2016). Animal study by Akinrinade et al. (2015) reported that excessive aluminium intake may induce the progression of cell deaths which leads to inhibition of AChE. Besides, other study by Phyu and Tangpong (2014) on sensitivity of AChE to environmental pollutants reported that other heavy metals such as lead may cause inhibition due to free radicals released as studied on painters and rat (Khan et al., 2009; Tsakiris, 2000). An alternative explanation also includes that fact that more than 90% of the comparison group were living within 5 km of intensive agricultural area. There may be the possibility that there are environmental cross-contamination that may have affected the blood cholinesterase level of the comparison groups living in Kuantan, Pahang to be depressed.

Neurobehavioral performance testing using NCTB is a method for early detection of nervous system impairment due to any effect of neurotoxic chemical exposure on human being. Ismail et al. (2012) and Ross et al. (2010) had conducted a meta-analysis studies that focused on OP exposure among adult agricultural workers and pesticide applicators. These two studies reported deficits in motor skills and slower reaction times. They found consistent findings across many studies on impaired neurobehavioral function concerning cognitive functions following low-level exposures to OP.

Based on the results of the present study, there were significant link between occupational exposures to OP with the neurobehavioral performance of the respondents in the exposed group. Pursuit Aiming and Trail Making tests were the neurobehavioral batteries that were found to exhibit the effects. The standard scores for both tests showed that the exposed group were linked to significantly poorer performance when compared to the comparison group. The poorer performance indicates poor or impaired motor steadiness (Pursuit Aiming Test) and visual and motor coordination (Trail Making Test).

The results of this study is aligned with a previous study by London et al. (1997) that reported an association of OP exposure with NCTB Pursuit Aiming test among fruit farm workers. The study by London et al. (1997) were conducted on 163 male sprayers and 84 male non-spraying workers of fruit farms in South Africa. Like the present study, Farahat et al. (2003) also revealed that the Trail Making Test were one of the sub-tests of NCTB where the exposed group exhibited significantly lower performance than the control group after adjusting the confounders of age and education level. Both studies were cross sectional studies which determine the long term of OP exposure with the neurobehavioral outcomes. The study by Farahat et al. (2003) were performed among 52 male cotton crop workers in Turkey who were being compared with 50 unexposed male controls that were similar in age, socioeconomic class, and years of education.

In the present study, it was found that the blood cholinesterase level of the comparison group was lower than the exposed group. Despite that, even though it was may be due to environmental cross contamination, 91.1% of the cocoa farmers had blood cholinesterase level above the normal range. As for the association of blood cholinesterase level, there was no any significant correlation between the blood cholinesterase levels with any of the neurobehavioral performance test scores. This study had aligned findings with study by Farahat et al. (2003) where acetylcholinesterase was not significantly correlated with either neurobehavioral performance or neurological abnormalities.

Although there was no significant correlation between blood cholinesterase levels with any of the neurobehavioral performance test scores in this study, but through monitoring and assessment of blood cholinesterase level, the cocoa farmers were still at risk of having depressed blood cholinesterase level as some of them have blood cholinesterase level in the range of overexposure and serious over exposure. Occupational exposure does occur and during the questionnaire administration session with the cocoa farmers, many of them said that they felt burning sensation on their face when they sprayed the pesticides as the wind carried the sprayed pesticide through their direction. The cocoa farmers also have differing frequency of pesticide spraying activities for each phases of cocoa harvesting in which it ranged from one, two or four times in a month. A cross-sectional study such as the present study may not be able to full assess the extent of the exposures to pesticides at just one point of time.

It is also important to underline that there was no reported baseline reading of blood cholinesterase level among these cocoa farmers to enable comparison to be made. Baseline measurement of blood cholinesterase level among those handling organophosphate pesticides should be conducted prior to employment (Pesticide Management Education Programme, 1993). Thus, this study may provide data that is useful for the relevant administration to enable safety precautions or other implementations regarding the protection of farmer's health takes place.

Further analysis was performed on two of the NCTB subtests which were Pursuit Aiming and Trail Making tests. The most important factors affecting the scores of Pursuit Aiming Test were income and years of handling OP pesticides. Results showed that years of handling pesticides had an inverse relationship with Pursuit Aiming test score. These findings were aligned with the previous studies by Roldan-Tapia et al. (2005) and Kamel et al. (2003) as they reported decrement of neurobehavioral performance was significantly related with increasing years of working in agriculture field.

Income was one of the significant predictor factors as it has significant positive relationship with Pursuit Aiming test. In other words, the higher the income of the cocoa farmers, the higher the standard score of their Pursuit Aiming test. Previous study reported total income were one of the confounders that were associated with the outcome of neurobehavioral performance (Starks et al., 2012). Similar to the study by Marston (2013) which revealed that those who had low income may had problem with their neurobehavioral development as chronic stress due to low income can contribute to significant problems towards executive functioning, working memory, social comprehension and emotion regulation.

In addition, it was found that three variables which were the use of gloves, age and education level were the significant predictors of the Trail Making test. An increase of age was inversely linked with the standard score of the Trail Making test while an increase of education level and the use of gloves were linked to increased score of the Trail Making test. Impairment of neurobehavioral functions may elevate if gloves are not used or unsuitable type of gloves are used instead, as OP pesticides in formulation of solvents may enhance greater uptake by the skin and is difficult to be removed from the body (International Program on Chemical Safety (INCHEM), 2017). For age, this study on cocoa farmers were able to support previous findings which reported that when age increased, the standard score of Trail Making test will be significantly decreased. Younger group of respondents may perform well in this test, as human neurological functioning will deteriorate as age increases (Rohlman et al., 2007b). Based on the results obtained, an increase of the education level will also significantly increase the standard score of Trail Making test. This was consistent with the findings from Anger (2003), which presented significantly poorer scores among newly literate adult Nicaraguan males compared with non-neurotoxicant exposed Hispanic groups in the United States.

Conclusion

The findings of this study have shown that neurobehavioral assessment conducted have provided an insight into the early effects of OP pesticide exposures when results detected impairment of cocoa farmers' motor steadiness and visual motor coordination. The blood cholineterase level in this study have provided baseline health data for the relevant administration of these farmers and can be used by the relevant authority to encourage commitment of employers to protect workers through conducting medical surveillance, reviewing existing training modules, assist in the amendment of the existing guideline for use and provide adequate and suitable personal protective equipment for the use of the workers in order to fulfil the objectives of the Occupational Safety and Health Act.

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