Unity in Diversity and the Standardisation of Clinical Pharmacy Services

Editors: **Elida Zairina, Junaidi Khotib, Chrismawan Ardianto, Syed Azhar Syed Sulaiman, Charles D. Sands III and Timothy E. Welty**

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Unity in Diversity and the Standardisation of Clinical Pharmacy Services

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Organophosphate toxicity in red chili farmers, Ciamis, Indonesia

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ABSTRACT: The use of pesticides in Indonesia is increasing due to the socioeconomic situation in this country. This study was aimed to understand the impact of Personal Protective Equipment (PPE) to Organophosphate (OP) toxicity. Farmers planting red chili in Ciamis, Indonesia had been recruited, who were routinely and actively applying OP-containing pesticides. The blood chemistry, physical assessment, quality of life data, and OP level in red chili and ground were collected before and after the use of PPE. Thirty five farmers experienced tremors before and after the use of PPE. The impact of PPE used is significantly present in the hemoglobin, thrombocyte, erythrocyte sedimentation rate, lymphocyte, cholinesterase level and emotional function ($p < 0.05$). Nausea and dizziness decreased after the use of PPE. The residue of OP is present in the red chili and plant area. PPE can protect the red chili farmers in Ciamis, Indonesia from acute toxicity of OP.

1 INTRODUCTION

The use of pesticides in Indonesia is increasing due to the socioeconomic situation in this country. This study was aimed to understand the impact of Personal Protective Equipment (PPE) to organophosphate (OP) toxicity. Farmers planting red chili in Ciamis, Indonesia had been recruited, who were routinely and actively applying OP-containing pesticides. The blood chemistry, physical assessment, quality of life data, and OP level in red chili and ground were collected before and after the use of PPE. Thirty five farmers experienced tremors before and after the use of PPE. The impact of PPE used is significantly present in the hemoglobin, thrombocyte, erythrocyte sedimentation rate, lymphocyte, cholinesterase level and emotional function ($p < 0.05$). Nausea and dizziness decreased after the use of PPE. The residue of OP is present in the red chili and plant area. PPE can protect the red chili farmers in Ciamis, Indonesia from acute toxicity of OP.

2 METHODS

2.1 *Subjects*

We recruited 35 red chili farmers in Ciamis, West Java, Indonesia, who actively and routinely applied the organophosphate-containing pesticides during the planting session. The intervention was the PPE in use for one month, during which the farmers used organophosphate-containing pesticides. All participants signed the informed consent form according to the Ethical Approval of Ethics Committee of Universitas Ahmad Dahlan Number 011605113.

2.2 *Data*

The laboratory data, physical assessment, laboratory test and quality of life data were collected before and after the use of PPE for one month. The quality of life was measured using the Indonesian version of Short Formulary-36 (SF-36) questionnaire. The PPE included head cover, goggle glasses, mask, body cover, and boot shoes. Laboratory data was collected and assessed by Private Laboratory Company which has been accredited by National Standardization Agency.

The level of organophosphate in the red chili samples and ground were assessed using Gas chromatography method.

2.3 *Data analysis*

Paired sample T test was used to find the differences of physical assessment, laboratory test, and quality of life before and after the use of PPE for one month.

3 RESULTS AND DISCUSSIONS

Our study found that the use of PPE may influence the organophosphate toxicity, which is present in the forms of decrease of acute toxicity symptoms, laboratory data, and quality of life. Moreover, we also identified the profenovos concentration in the red chili.

Table 1 presents the farmers' characteristics in Ciamis, West Java, Indonesia. Our study recruited 35 red chili farmers who are mostly male's workers (94.3%). After the use of PPE, around 6 farmers did not participate in the study anymore. Most of their latest education was up to senior high school (62.9%) and their age mean was 42.23 years (SD 13.04). All of them experienced tremor both in the baseline and after PPE in use assessment. The significant increase was seen from the data of haemoglobin (14.7 to 15.3 g/dL), thrombocyte (282.3 to 314.8 uL), blood urea nitrogen (9.43 to 11.6 mg/dL), and creatinine serum (0.77 to 0.88 mg/dL) ($p < 0.05$). The significant decrease was seen from the data of lymphocyte (36.6 to 33.2%), cholinesterase (8.32 to 7.92 Ku/L) and erythrocyte sedimentation rate for first hour (9.71 to 4.55), for second hour (17.9 to 13.1) ($p < 0.05$).

The use of PPE in our study can increase the hemoglobin and thrombocyte, moreover may decrease the lymphocyte, significantly. These results are in line with previous study which presented that the hemoglobin level of the control group was higher than the organophosphate-poisoned group (Hundekari et al. 2013).

The report of impaired of platelet function was found in previous case report of children exposed to home-made shampoo-contained organophosphate (Sadaka et al. 2011). Organophosphate could modify the antioxidant defense capability of person exposed by pesticides. Furthermore, the susceptibility of subjects to oxidative stress could be affected. This situation may cause the change of erythrocyte and lymphocyte which presented the stability of oxidative reaction and antioxidant defense capability (Banerjee et al. 1999). The decrease of erythrocyte sedimentation rate after the use of PPE showed the inflammation in the body, which may be caused by the organophosphate poisoning in the body. This result is supported by a previous case report which presented the change of complete blood count (Rizos et al. 2004).

Regarding to the renal function, after the PPE in use, the creatinin serum and BUN level increased. Our study results cannot confirma previous study that the use of PPE may protect the renal function (Rubio et al. 2012). This could be caused by the short use of PPE and other confounding variables may influence renal function, which we could not control during the study. The investigators have been encouraging the use of PPE; however, the

farmers did not employ the PPE properly. The farmers used inappropriate equipment to protect themselves, such as common mask, repeated use of the clothes and did not use goggle glasses and boot. The farmers feel that PPE is annoying their activity. These reasons are also present in another study (Lu 2009). The suboptimal use of PPE could cause the organophosphate exposure to keep going on, thus the renal function tests and cholinesterase level in the second assessment decrease. These results are similar to the previous study in Thailand which presented that the long exposure of organophosphate may keep the low level of cholinesterase (Wilaiwan & Siriwong 2014).

Table 1. Farmers' characteristics in Ciamis, West of Java, Indonesia.

Demography characteristics	Number	%
Sex		
Male	33	94.3
Female	2	5.7
Last Education		
No school	1	2.9
Elementary	10	28.6
Up to High School	22	62.9
Bachelor	2	5.7
Marriage status		
Married	31	88.6
No	4	11.4
Age	Mean: 42.23	SD: 13.04
Health characteristics	**Number**	**%**
Blood Pressure Pre PPE		
Normal	23	65.7
Hypertension stage 1	10	28.6
Hypertensionstage >2	2	5.7
Post PPE		
Normal	10	28.6
Hypertension stage 1	15	42.9
Hypertension stage >2	4	11.5
Number of disease's history 1	12	34
More than 1	23	66
Tremor		
Yes	35	100
No	29	100
Mean ± SD		
	Pre PPE	**Post PPE**
Body weight (kg)	57.63 ± 8.5	46.57 ± 26.77
Hemoglobine (g/dl)*	14.74 ± 1.56	15.3 ± 1.46
Erythrocyte (million/ul)	4.62 ± 0.49	5.19 ± 0.40
Hematocrite (%)	45.44 ± 3.2	45.10 ± 4.38
Leucocyte (uL)	7.61 ± 1.44	8.15 ± 2.25
MCV (iL)*	87.26 ± 6.2	86.7 ± 6.86
MCH (pg)	28.69 ± 2.4	29.14 ± 2.48
MCHC (pg)*	33.33 ± 33.56	31.03 ± 1.10
RDW (%)	13.20 ± 1.2	13.5 ± 1.4
Thrombocyte (uL)	282.34 ± 59.8	314.6 ± 13.47
Lymphocyte (%)*	36.60 ± 8.2	33.28 ± 6.28
Cholinesterase (Ku/L)*	8.32 ± 2.3	7.92 ± 1.99
Blood Urea Nitrogen (mg/dl)*	9.43 ± 2.4	11.6 ± 3.1
Creatinine serum (mg/dl)*	0.77 ± 0.08	0.88 ± 0.15*
Erythrocyte sedimentation rate, first hour*	9.71 ± 1.8	4.55 ± 7.1
Erythrocyte sedimentation rate, second hour*	17.97 ± 3.3	13.17 ± 15.07

* Significant differences between pre and post PPE in use ($p < 0.05$).

All the patients experienced tremor as the symptoms of chronic toxicity of organophosphate. The inactivation of acetylcholinesterase by organophosphate may cause accumulation of acetylcholinesterase in muscarinic, nicotinic and nervous system sites. Our study results are in line with a previous study in India that found around 58% patients to experience acute organophosphate poisoning with tremor (Reji et al. 2016). Another study in New York also presented the neurotoxicity symptoms of male applicators-exposed by organophosphate (Stokes et al. 1995).

Specifically, we also explore the farmers' condition that experienced abnormality of full blood count. Before the use of PPE, the abnormality of haemoglobin, erythrocyte, hematocrite, leucocyte, thrombocyte and cholinesterase are shown in 11.4%, 31.4%, 2.8%, 5.7%, 5.7% and 8.5% farmers, respectively. After the use of PPE, the abnormal proportion of full blood count decreased. We found one farmer with low level of cholinesterase and low level of hemoglobin. Moreover, there are two farmers with low cholinesterase and normal complete blood count level. The three farmers with low level of cholinesterase do not use PPE during the application of organophosphate-containing pesticide, work in wide area, long duration of application of organophosphate-containing pesticide and wash their clothes mixed with others. After the use of PPE, the farmers with low level of cholinesterase on baseline period experience the increase of cholinesterase level. The increase of cholinesterase level is followed by the increase of hemoglobin.

Two farmers with high level of leucocyte do not use PPE during the pesticide application and have long duration of application of organophosphate-containing pesticide. After the use of PPE, there are two farmers with the decrease of leucocyte level followed by the decrease of the cholinesterase level. Four farmers with low level of thrombocyte also did not use PPE during the application and had long duration of application of organophosphate-containing pesticide.

Table 2 presents the characteristics of organophosphate-containing pesticides, the use of PPE and the characteristics of acute toxicity symptoms. According to the use of organophosphate-containing pesticides, the farmers applied the pesticides around 12 hours before the assessment, both in before and after the use of PPE. They applied the organophosphate-containing pesticides for 1–2 times/week.

Table 2. Characteristics of organophosphate-containing pesticides application, the use of PPE and acute toxicity symptoms.

Application of organophosphate-containing pesticides	Mean	*SD*
Hours before assessment		
First assessment	12	*7.9*
Second assessment	12.1	*12.2*
Wide area (m^2)	3800	*119.8*
Duration (month)	74	*7.2*
Frequency/week	1.54	*0.7*
Hours of pesticide application		
First assessment	3.24	*1.60*
Second assessment	2.69	*1.17*
The use of personal protective equipment	**Number**	**%**
Application of organophosphate-containing pesticides	**Mean**	***SD***
First assessment		
Complete PPE	16	*46*
Incomplete PPE	19	*54*
Second assessment		
Complete PPE	0	*0*
Incomplete PPE	29	*100*
Reason for not using PPE		
Discomfort	5	*15*
Unavailability	4	*11*
No explanation	26	*74*
Wash the clothes after using pesticides First assessment		
Separated from other clothes	28	*80*
Mixed with other clothes	7	*35*
Second assessment		
Separated from other clothes	23	*79*
Mixed with other clothes	6	*20*

Acute symptoms after using pesticides First assessment		
Nausea	**2**	*5.7*
Nausea and Vomiting	**1**	*2.9*
Dizziness	**3**	*8.6*
Nausea and Dizziness	**28**	*80*
Second assessment		
Nausea	**1**	*2.9*
Nausea and Vomiting	**1**	*2,9*
Dizziness	**3**	*8.6*
Nausea and Dizziness	**20**	*57*
Behavior toward the symptoms		
Nothing to do	**17**	*49*
Buy medication	**8**	*29*
Find physician	**4**	*11*
Drinking milk	**2**	*5*

However, the duration of organophosphate-containing pesticide application decreased from around 3 hours to 2 hours after the PPE in use. There were no farmers using complete PPE. After using the PPE, the farmers who washed their clothes separated from other clothes decreased from 80% to 17%. After the use of PPE, the proportion of workers who experienced nausea and dizziness decreased from 80% to 57%. Moreover, around 49% subjects did not find medication to neutralize the symptoms.

The acute toxicity symptoms of nausea and dizziness decreased after the use of PPE. The decrease of these symptoms shows the tolerance mechanism of the body. The tolerance mechanism is defined by the decrease of response at certain dose following the repeated exposure, then need higher dose to reach similar effect (Vashista and Berrigan, 2017).

According to the symptoms of acute toxicity, the awareness of farmers to find medication is quite low. Most of them prefer to let the symptoms gone and did not recognize that they experienced the acute symptoms of organophosphate. The awareness of using PPE is also low due to the uncomfortable feeling of using the equipment and unavailability of the equipment. Even though the investigator already promoted the important of using PPE and separately wash their clothes, but the proportion of using complete PPE increase and the use of complete PPE decrease. Moreover, the proportion of separately wash the clothes decrease. These results are in line with previous study which stated that not all farmers were aware about using the PPE (Perry et al. 2000; Yassin et al. 2002).

Table 3 presents the quality of life score before and after the use of PPE. In general, the scores of qualities of life domain increased except for physical domain, pain and general health. The significant increase was shown in emotional health related to physical role and the significant decrease was shown in the pain domain.

In general, the scores of quality of life domains increase, except for physical function, pain, and general health. A previous study in Iran described that the farmers' quality of life was lower than the control group ($p > 0.05$). Only mental health of the farmers group and control group was significant different. However, the scores of quality of life domains in our study are higher than the previous study in Iran (Taghavian et al. 2016). According to the study design, the study in Iran did not measure the QoL differences before and after the use of PPE, hoewever, the author compared the QoL in farmer group and control group.

Table 3. Farmers QoL before and after the PPE in use.

Domain	Before PPE in use (x ± SD)	After PPE in use (x ± SD)
Physical Function	73.5 ± 23.1	66.9 ± 25.3
Role limitation-physical	47.1 ± 31.8	50.0 ± 29.5
Role limitation-emotion*	47.8 ± 33.9	66.1 ± 36.4
Energy	66.5 ± 11.5	68.9 ± 13.8
Emotional Function	77.7 ± 15.4	77.0 ± 16.6
Social Function	63.4 ± 17.9	66.7 ± 19.1
Pain*	64.8 ± 19.1	55.8 ± 19.4
General Health	64.1 ± 10.5	62.7 ± 10.1

* Significant difference ($p < 0.05$).

Table 4 lists the pesticides concentration in the red chili and plantation ground. It can be seen that the concentrations of profenovos were higher than 0, 33 mg/Kg in the red chili. According to the government rule of Joint Decree of Health Minister and Agriculture Minister Number: 881/MENKES/ SKB VIII/1996 and 711/Kpts/TP.270/8/96, 22 August 1996 about Maximum Limit of Farmers Products. The maximum limit of profenovos pesticide residues in red chili was 0, 5 mg/kg. Two of the four samples of red chili contained profenovos pesticides above the threshold set by the government.

Table 4. Residue of organophosphate in red chili and ground.

		Average of concentration (mg/Kg)	
Sample group	Pesticide	Red chili	ground
A	Diazinon	Not detected	Not detected
	Parathion	Not detected	Not detected
	Ethion	Not detected	Not detected
	Profenofos	0,61	Not detected
	Malation	Not detected	Not detected
	Chlorpyrifos	Not detected	Not detected
B	Diazinon	Not detected	Not detected
	Parathion	Not detected	Not detected
	Ethion	Not detected	Not detected
	Profenofos	0,39	Not detected
	Malation	Not detected	Not detected
	Chlorpyrifos	Not detected	Not detected
C	Diazinon	Not detected	Not detected
	Parathion	Not detected	Not detected
	Ethion	Not detected	Not detected
	Profenofos	0,48	Not detected
	Malation	Not detected	Not detected
	Chlorpyrifos	Not detected	Not detected
Tanjung (PHT)	Diazinon	Not detected	Not detected
	Parathion	Not detected	Not detected
	Ethion	Not detected	Not detected
	Profenofos	0,55	Not detected
	Malation	Not detected	Not detected
	Chlorpyrifos	Not detected	Not detected

*** Limit of Detection for organophosphate analysis are: diazinon 3,84 μg/kg, parathion 0,82 μg/kg, ethion 2,76 μg/k g, profenofos 0,80 μg/kg, malathion 0,50 μg/kg, danchlorpyrifos 0,33 μg/kg.**

Profenofos insecticide residues found in chili enters the body through the mouth, it may influence the human health. The impact on consumers is generally in the form of which chronic poisoning is not directly perceived. Symptoms of poisoning is a new look after a few months or years later (Dalimunthe et al. 2015). Organophosphate pesticide residue was using Gas Chromatography, where the Limit of Detection (LoD) for organophosphate analysis are 3,84 µg/kg, 0,82 µg/kg, 2,76 µg/kg, 0,80 µg/kg, 0,50 µg/kg, and 0,33 µg/kg for diazinon, parathion, ethion, profenovos, malathion and chlorpyrifos, respectively. Thus, the results of the analysis which were not detected had no possibility of its existence. None of the organophosphate pesticides except profenovos were applied during the whole period of the experiment in this treatment. Profenovos pesticides were not detectable in the ground sample. Studies conducted EPA (Environmental Protection Agency) 1999 profenofos had a degradation time of 7–8 days.

The degradation of pesticide was influenced by many factors, including application factors (times, rate, position etc.), pesticide properties (toxicity, persistence, volatility etc.) and weather conditions (temperature, humidity, wind, and photo effect) and microorganisms, etc. In this study, the times and rate of application, and rain were primary reasons for pesticide disappearance.

In addition, the effect of rains on degradation of organophosphates was probably less than pyrethroids (Zhang et al. 2007). Organophosphate physical-chemistry properties are also expected to affect the concentration of some organophosphates which are not detected by gas chromatography. Based on the analysis performed, the operational conditions of gas chromatography using column temperature 220°C, whereas some organophosphates only have a boiling point of not more than 220°C (Ekadewi, 2007). This causes some organophosphates already evaporated or broken first before reaching the detector gas chromatography.

Our study has limitation in sample size of study. We cannot fulfill the sample size criteria, due to the limited number of farmers who wanted to participate in this study. Further study need to be conducted to understand the association between variables in this study. Moreover, we could not control the use of PPE during one month, thus the results of this study still being confounded by particular variables.

4 CONCLUSION

The use of PPE may decrease acute toxicity symptoms of organophosphate, influence the full blood count level and improve the farmers' quality of life.

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