Risk Factors for Organophosphate Pesticide (OP) Exposure among Indonesian and South Australian Migrant Farmworkers and the Impact of an Intervention to Reduce Exposure

Suratman

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School of the Environment

Faculty of Science and Engineering

Flinders University

South Australia

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Abstract

Organophosphate compounds are the most widely used pesticides in the world. Organophosphate pesticides (OPs) contribute to mortality and morbidity in farmworkers through acute or chronic pesticide-related illnesses. While the factors that increase OP exposure and cause adverse health effects among farmworkers in developing and developed countries have been investigated in the past, there is a paucity of relevant research in Indonesia and Australia.

This study consisted of quasi and true experimental designs. A quasi-experimental design is defined as a design that is similar to an experimental design but lacks a key ingredient, random assignment. This research design is sometimes called as non-randomised pre-post intervention studies. This design involves selecting groups, upon which a variable is tested, without any random pre-selection processes. After this selection, the experiment proceeds in very similar way to any other experiment, with a variable being compared between different groups, or over a period of time. The quasi-experimental study investigated benefits arising from short educational intervention, either delivered using a group communication among Indonesian farmworkers or one-on-one approach among South Australian (SA) migrant farmworkers. Specifically, the study assessed knowledge of adverse effects of OPs and self-protection from OP exposure, perceptions about OP exposure, field practices in handling OPs to reduce OP exposure, and activity levels of plasma cholinesterase (PChE) as a biomarker of exposure to OPs and erythrocyte acetylcholinesterase (EAChE) as a biomarker of toxicity in whole blood samples. Data collection used an interviewer-administered questionnaire and collection of a fingerprick blood sample before and following the intervention. Fingerprick blood samples were assessed immediately using Test-mate ChE field kit instrument to

measure EAChE and PChE activities. Meanwhile, the true experimental study examined whether the interaction between pralidoxime (pyridine-2-aldoxime methochloride) solution in saline leads to changes in PChE activities inhibited by OPs using fresh plasma samples in field measurements as a method to estimate percent inhibition of PChE activities due to OP exposure. Blood samples were centrifuged to separate plasma. Plasma samples were then divided into two portions. One 8µL portion was mixed with 2µL pralidoxime solution in saline and the other portion was mixed with 2µL saline solution. PChE of each sample was analysed using the same field kit.

This study was conducted at two research sites, at Dukuhlo Village in Brebes Regency, Indonesia and the suburb of Virginia, South Australia. In Indonesia, 30 of 52 Indonesian farmworkers working and living at the village were randomly selected. On the other hand, due to many difficulties in recruiting research participants in Australia, a snowball sampling method by asking research participants to nominate another farmworker with the same trait as our next participant was used to select seven SA migrant farmworkers resulting in a sample size of seven farmworkers. Nominate another farmworker with the same trait means proposing another farmwoker who has the same characteristics to be a research participant in accordance with inclusion criteria, namely male, had to be employed in farm work within the past 3 months. The ethnicity of the SA migrant farmworkers was Vietnamese. All those research participants were involved both study designs. In addition, twenty-four venous blood samples from random blood donations collected once from the Australian Red Cross Blood Service (ARCBS, Adelaide, SA) were added as a third sampling group in the true experimental study.

Results of the educational intervention showed statistically significant improvements in scores of knowledge, perceived susceptibility, perceived severity, perceived benefits, and perceived barriers, except for cues to action among Indonesian farmworkers after being adjusted for level of education and years working as a farmworker. In contrast, SA migrant farmworkers had statistically insignificant improvements in almost all measured variables, except for knowledge about adverse effects of OPs. Generally, the intervention did not significantly change field practices in both groups. However, some self-reported significant behavioural improvements in handling OPs occurred among Indonesian farmworkers, for example, not touching crops after OP application, not spraying OPs against wind direction, avoiding spray drift when applying OPs and ensuring to not affect other people by over applied spray drift when applying OPs. In addition, the proportion of farmworkers who were suffering from OP-related symptoms slightly decreased from 67% in pre intervention to 63% in post intervention. In general, the field practices of SA migrant farmworkers in post intervention remained constant compared with pre intervention. A group communication was more effective in improving knowledge, perceptions, and some aspects of field practices in handling OPs compared with the one on one intervention. Notwithstanding, the differences in EAChE and PChE activity levels between pre and post intervention could be related to the time elapsed since last exposure and not to the intervention performed. Furthermore, the results of the true experimental study demonstrated that PChE re-activation ranging from 36% to 39%. The estimation of percent inhibition of PChE activities in fresh plasma samples due to OP exposure among these three groups showed that the highest inhibition occurred among SA migrant farmworkers, approximately 33%, otherwise Indonesian farmworkers and ARCBS were similar, approximately 28%.

Among Indonesian farmworkers, factors of knowledge, perceived susceptibility of OP exposure, perceived severity of adverse health effects due to OP exposure, perceived benefits of personal protective equipment (PPE) use and field practices in handling OPs played an important role in increasing OP exposure. Meanwhile, perceived barriers to PPE use, following OPs safety procedures, and cues to action were identified as important factors in increasing OP exposure among SA migrant farmworkers. In addition, the use of group communication was more effective in improving farmworkers' knowledge and perceptions compared with the individual approach. Notwithstanding, the effect of different periods between OP application and blood collection might also influence the differences of these results between both study groups. Provision of appropriate equipment and long-term educational intervention linked to workplace conditions was needed to improve their knowledge, perceptions, and work practices to reducing adverse effects due to OP exposure. Pralidoxime assay can be a useful exposure measurement tool to use under field conditions.

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Statement of Authenticity

I certify that this thesis does not incorporate without acknowledgment and any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Suratman

7th December 2015

Statement of Co-Authorship

The following people contributed to the publication of the work undertaken as part of this thesis. The co-authors are listed in the order that the co-authored publications appears in the thesis.

Dr. Kirstin Ross

Dr. Kateryna Babina

Associate Professor John William Edwards

All above listed contributions equated to no more than 25% of the work necessitated for publication of research manuscripts.

Suratman

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Publications

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Note to Readers: This thesis is based on published papers or manuscripts under review, therefore some repetition between chapters occurs.

Comments from examiners for this thesis have been incorporated in the text, including those chapters representing manuscripts that have been published or accepted for publication prior to examination. Unedited texts are in the published for as cited.

Chapter 1. Literature Review

Organophosphate Pesticides Exposure among Farmworkers: Pathways and Risk of Adverse Health Effects

Suratman[1,2], **John William Edwards**[1], **Kateryna Babina**[1]

[1.] Health and Environment Group, School of the Environment, Faculty of Science and Engineering, Flinders University, Adelaide, SA, Australia.

[2.] School of Public Health, Faculty of Health Sciences, Jenderal Soedirman University, Kampus Karangwangkal, Purwokerto 53122, Indonesia.

Keywords

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Abstract

Organophosphate (OP) compounds are the most widely used pesticides with more than 100 OP compounds in use around the world. The high-intensity use of OP pesticides contributes to morbidity and mortality in farmworkers and their families through acute or chronic pesticides-related illness.

Many factors contributing to adverse health effects have been investigated by researchers to determine pathways of OP pesticide exposure among farmers in developed and developing countries. Factors like wind/agricultural pesticide drift, mixing and spraying pesticides, use of personal protective equipment (PPE), knowledge, perceptions, washing hands, taking a shower, wearing contaminated clothes, eating, drinking, smoking, and hot weather are common in both groups of countries.

Factors including low socioeconomic status areas, workplace conditions, duration of exposure, pesticide safety training, frequency of applying pesticides, spraying against the wind, and reuse of pesticide containers for storage are specific contributors in developing countries whereas housing conditions, social contextual factors, and mechanical equipment were specific pathways in developed countries.

This paper compares existing research in environmental and behavioural exposure modifying factors and biological monitoring between developing and developed countries. The main objective of this review is to explore the current depth of understanding of exposure pathways and factors increasing the risk of exposure potentially leading to adverse health effects specific to each group of countries.

1. Introduction

Pesticide use significantly increases from year to year particularly in developing countries. Pesticides are beneficial in food production, improving crop yields and efficiency of food production processes, reducing the cost of food, and providing a high-quality produce for consumers. Approximately 40 % of food production around the world is lost every year due to weeds, pests, and diseases. Crop losses would be doubled if farmworkers did not apply pesticides on their crop. In addition, safe domestic use of pesticides also have some benefits to control pests relevant to public health and infrastructure (termites, roaches, ants, rats, and other pests) (Delaplane, 2000; Ghatax & Turner, 1978; Jeyaratnam, 1990).

Organophosphates (OP) are among the most widely used agricultural chemicals. More than 100 OP compounds are known and have been used in most countries around the world (Eddleston et al., 2005).

Agricultural workers are the population at most risk of exposure to OP pesticides. The aim of this paper is to review exposure pathways prior to the design of a study to assess ways to reduce OP exposure. The novelty of this review article is a comparison of environmental and behavioural factors including biological monitoring between developing and developed countries based on previous studies to determine specific pathways of OP pesticides exposure and factors affecting the risk of increased exposure leading to adverse health effects.

2. A Brief History of OP Compounds

OP compounds have been used for a long time to protect their crops from insect attacks. De Clermont and Moschnine discovered tetraethyl pyrophosphate (TEPP) as the first OP cholinesterase inhibitor in 1854 (Nurulain, 2011, 2012;

Soltaninejad & Shadnia, 2014). From 1934 to 1944, Schrader developed parathion, paraxon, tabun, sarin, and soman as nerve agents (Nurulain, 2011, 2012; Soltaninejad & Shadnia, 2014). Parathion was developed and introduced to the market for the first time in 1943 and is still being used on a wide scale today (Delaplane, 2000; Taylor et al., 2007).

After World War II, the use of OP pesticides in agriculture and public health rose significantly (Squibb, 2002). Most people used OP pesticides to control pests from the 1950s to the 1960s. The Cyanamid Company introduced malathion in 1950 (Soltaninejad & Shadnia, 2014; Taylor et al., 2007). In 1952, dichlorvos, trichlorfon and diazinon were developed (Casida & Quistad, 1998). Meanwhile, another nerve agent, VX, was developed in 1958 (Soltaninejad & Shadnia, 2014). From 1961, VX was mass-produced to be among the chemical warfare agents used by the military in Iraq (Soltaninejad & Shadnia, 2014). In the late 1950s, OP use rose dramatically in agriculture and horticulture (Department of Labour of New Zealand, 2000).

3. Pesticide Forms

Most OP are used as insecticides, with a few used as a fungicides, herbicides, or rodenticides. This group of pesticides is available in powder, liquid concentrate, or granules. All forms are susceptible to hydrolysis and oxidation. Moisture and sunlight play an important role in the environmental transformation process (Costa, 2008; Fenske & Edgar W. Day, 2005). Degradation of these compounds in the environment is fast. For example, malathion, chlorpyrifos, and diazinon degrade rapidly, with half-lives, respectively, ranging from 1 to 5 days, from about 2 to 14 days, and from 16 to 103 days at 25°C (Porto et al., 2011; Costa, 2008; Qiao, 2010; Starner et al., 1999).

Exposure to OPs in humans can occur via ingestion, inhalation, and dermal absorption. Uptake through the skin may be significant, because of the lipophilic nature of these compounds. Biotransformation of OPs occurs through three main reactions, namely, oxidation, transferase reaction, and hydrolysis (Kaloyanova & Batawi, 1991). Toxic effects of OPs occur through inhibiting acetylcholinesterase (AChE) (generally known as cholinesterase (ChE)) enzyme activity, inhibiting neuropathy target esterase (NTE), and releasing the alkyl groups attached to the phosphorous atom and the alkylation of macromolecules, including RNA and DNA (Costa, 2008; Kaloyanova & Batawi, 1991).

Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)-phosphorothionate] is one of the most widely used OP pesticides (Risher & Navarro, 1997; Smegal, 2000). Chlorpyrifos can enter the body through the gastrointestinal tract, skin, or inhalation (Risher & Navarro, 1997; Smegal, 2000). Chlorpyrifos experiences bio-activation to chlorpyrifos oxon in the liver through the cytochrome P-450 mediated desulfurization and then undergoes hydrolysis to 3,5,6-diethylphosphate and trichloro-2-pyridinol (TCP) (Qiao, 2010).

Chlorpyrifos has a short biological half-life, approximately 18 h in plasma and about 62 h in fat tissue (Qiao, 2010). However, due to their very wide use, chlorpyrifos metabolites are commonly found in human tissue (fat tissue). Excretion of chlorpyrifos occurs mainly through urine. Chlorpyrifos oxon is the active metabolite that causes toxic effects. The oral LD_{50} of chlorpyrifos in rats ranges between 82-270 mg/kg (Qiao, 2010). Immediately after exposure, PChE activity is more inhibited than EAChE (Lotti, 1995). The half-life of PChE recovery after OP exposure was about 12 days and complete recovery has been reported to occur after about 50 days (Mason, 2000). This contrasts with complete recovery of EAChE (attaining unexposed activity) after about 82 days, shorter than the normal life-span of erythrocyte (about 120 days) (Mason, 2000). Recovery from mild inhibition has been shown to be about 1-3 days whereas recovery from moderate inhibition is 1-2 weeks (Workplace Health and Safety Queensland, 2012).

OP poisoning occurs when OP compounds enter a human body through dermal, gastrointestinal, or respiratory system. The principle mechanism of action of OP pesticides is ChE inhibition. Acetylcholine (ACh) is an excitatory neurotransmitter. ChE breaks down ACh into choline and acetic acid in synaptic cleft and thus prevents over-stimulating post-synaptic nerves, muscles, and exocrine glands (Heide, 2007; Marrs, 2001).

OP inhibits ChE activity by occupying and blocking the location where the ACh attaches to the ChE. As a result, excessive amount of ACh accumulate at the skeletal neuromuscular junction and synapses and cause over-stimulation of post-synaptic nerves, muscles, and exocrine glands (Heide, 2007).

Specific cholinesterases, known as red blood cell Acetylcholinesterase (RBC-ChE), are in the nerve ganglion synapse and erythrocytes, whereas non-specific cholinesterases, known as butyrylcholinesterase (BuChE) or plasma cholinesterase (PChE), are found mainly in plasma and the liver (Marrs, 2001).

4. Intrinsic Factors Increasing Susceptibility to OP Effects

Two intrinsic factors that can increase susceptibility to OP pesticides are ChE and paraoxonase (PON1) (WHO, 2006) activity levels.

4.1. ChE

ChE is the target enzyme of OP in central and peripheral nervous systems. Lower ChE activity increases susceptibility to OP exposure (Gonzalez et al., 2012).

A study by Sözmen et al. (2002) assessing BuChE activity among 28 OP-poisoned patients and 66 healthy volunteers showed that BuChE activity was 50% lower (2276±738 U/L) in patients than (5037±1553 U/L) in controls ($p<0.01$). Another study among OP-exposed agricultural pesticide handlers in Washington State recruited during the period of the 2006 and 2007 spray seasons (Hofmann et al., 2009) revealed that all of the research participants had significantly lower BuChE activity during the OP spray season compared to preseason levels (n=163; $p<0.001$).

4.2. Paraoxonase (PON1)

Paraoxonase (PON1) is a polymorphic enzyme synthesized in the liver and transported, along with a high-density lipoprotein in plasma that plays an important role in hydrolyzing the active metabolites of OP compounds such as diazinon, chlorpyrifos, and parathion (Costa et al., 2012; Hernández et al., 2003; La Du et al., 1999). Paraoxonase is one of the two genes that is known to increase susceptibility to OP pesticides. Paraoxonase, further modifies an individual's susceptibility to OP toxicity (i.e. individuals can vary 11-fold in the ability to inactivate the OP pesticide parathion, depending on which gene for this enzyme they carry (Brophy et al., 2001). A study by Sözmen et al. (2002) investigating PON1 activity among 28 OP-poisoned patients and 66 healthy volunteers showed that PON1 activity was 30% lower (114.2 ± 67.4 nmol/mL/min) in patients than (152.9 ± 78.9 nmol/mL/min) in controls ($p<0.05$). Similarly, Ellison et al. (2012) in Egypt demonstrated that PON1 55 and PON1 192 genotypes had a significant influence to PON1 activity among agricultural workers exposed to chlorpyrifos. PON1 phenotype is an important factor of susceptibility to OP toxicity. Among 163 OP-exposed agricultural pesticide handlers in Washington State there was significant association between lower levels of plasma PON1 activity and greater BuChE inhibition (Hofmann et al., 2009). Costa et al.

(2012) found PON1 to be an important factor of OP-pesticide toxicity among rats and mice, especially in the toxicity of diazinon and chlorpyrifos oxon.

5. Monitoring of OP Pesticides Exposure

Agricultural workers are frequently occupationally exposed to OP pesticides in occupational setting. Common methods to monitor exposure to OP pesticides use data collected from environmental measurements and/or in biological samples.

5.1. Environmental Monitoring

The aim of the environmental monitoring is to measure hazardous materials in environmental media, such as air, surfaces, household dust, water, food, and soil. It is particularly important when a single exposure route has been identified (Needham et al., 2005). According to Hoppin et al. (2006), surface sampling indoors can be measured using methods of deposition pad samples, a wipe sampling technique, or a vacuum technique. Air samples can be measured using high-volume samplers. Meanwhile, a duplicate plate method is used to analyse OP pesticides exposure in food samples.

Some studies had demonstrated the existence of OP in the environment. Simcox et al. (1995) measured OP compounds (azinphosmethyl, chlorpyrifos, parathion, and phosmet) in household dust and soil samples collected from children's play areas near operating apple or pear orchard. The samples were extracted and analysed by gas chromatography/mass selective detection. As many as 62% of household dust samples contained these four OP compounds, and 66.7% of the farm homes were found at least one OP in quantities above 1000 ng/g. %s were % of the samples that contained OPs. The detection limit of household dust sampling ranged from non-detectable to 17,100ng/g. There were no interferences. For reference families, OP concentrations ranged from nondetectable to 820 ng/g. Another study by Gordon et al. (1999) revealed that chlorpyrifos and diazinon had been detected in all sample media (indoor and outdoor air, house dust, and foundation soil. Chlorpyrifos were found in floor dust (88%), indoor air (65%), personal air (17%), yard soil (31%), foundation soil (48%), and outdoor air (ng/m^3) (10%). Meanwhile, diazinon had been identified in floor dust (53%), indoor air (63%), yard soil (18%), foundation soil (37%), and outdoor air (ng/m^3) (21%).

5.2. Biological Monitoring

According to Berlin et al. (1980), biological monitoring means "*the measurement and assessment of workplace agents or their metabolites either in tissues, secreta, excreta, expired air or any combination of these to evaluate exposure and health risk compared to appropriate reference*".

The National Research Council classified biological monitoring into three types as follows: (1) biological monitoring of exposure (i.e., measuring the dose of pesticides absorbed by the human body and identifying its metabolites in urine after exposure); (2) biological monitoring of effect (i.e., blood cholinesterase testing); and (3) biological monitoring of susceptibility (i.e., PON1 status) (National Research Council, 2006).

Biological monitoring has an important role in identifying any substances that accumulate in the human body exposed to chemicals, especially pesticides through measurement of biomarkers in biological samples. Biological monitoring is the best method to analyse to what extent a farmworker has been exposed to pesticides due to multiple pathways by which exposure can occur (Edwards, 2007; WHO, 1993).

Table 1 presents some previous studies regarding biological monitoring (AChE, PChE, PON1, metabolites) of OP-pesticide exposure in developing (Afriyanto, 2008; Catano et al., 2008; Cecchi et al., 2012; Dasgupta et al., 2007; Jintana et al., 2009; Kashyap, 1986; Panuwet et al., 2009; Panuwet et al., 2008; Phung et al., 2012; Shomar et al., 2014) and developed countries (Benmoyal-Segal et al., 2005; Bouvier et al., 2006; Costa et al., 2012; Furlong et al., 2005; Gonzalez et al., 2012; Grandjean & Landrigan, 2014; Hernández et al., 2003; Hofmann et al., 2009; Koch et al., 2001; La Du et al., 1999; Lopez-Granero et al., 2014; Marsillach et al., 2011; Nomura et al., 1986; Rohlman et al., 2011; Sanchez-Santed et al., 2004; Tromm et al., 1992). Generally, some biomarkers (AChE, PChE, and metabolites) and symptoms of OP-pesticide exposure have been investigated by researchers in both groups of countries. However, researchers in developing countries have generally not measured PON1 in their studies. This is completely different from developed countries, where most studies have linked these biomarkers with neuro-behavioural outcomes (Table 1).

Table 1: Biological monitoring of OP-pesticide exposure studies in developing and developed countries.

Study Sites	References	Biological monitoring related to risk factors						
		AChE	PChE	PON1	Metabolites	Health Effects	Environmental Factors	Behavioural Factors
Developing Countries	Shomar et al. (2014)	-	-	-	o	-	-	o
	Panuwet et al. (2008)	-	-	-	X	-	-	X
	Afriyanto (2008)	X	-	-	-	-	o	X
	Jintana et al. (2009)	X	X	-	-	-	-	X
	Dasgupta et al. (2007)	X	-	-	-	^	-	-
	Cecchi et al. (2012)	X	X	-	-	-	X	o
	Kashyap (1986)	X	X	-	-	o	-	o
	Panuwet et al. (2009)	-	-	-	X	-	-	-
	Phung et al. (2012)	-	-	-	X	-	-	-
	Catano et al. (2008)	-	X	-	-	X	-	X

X = Significant (*p*<0.05) in bivariate analysis; XX= Significant (*p*<0.05) in multivariate analysis; o = Factor was not statistically examined;
^ = Factor was examined, no significant association; ~ = Factor was examined, no information regarding association test; - = Factor was not investigated

(Table 1: Continued)

Study Sites	References	Biological monitoring related to risk factors						
		AChE	PChE	PON1	Metabolites	Health Effects	Environmental Factors	Behavioural Factors
Developed Countries	Gonzalez et al. (2012)	X	X	X	-	-	-	-
	Benmoyal-Segal et al. (2005)	X	-	X	-	-	-	-
	Hofmann et al. (2009)	-	X	X	-	-	-	-
	La Du et al. (1999)	-	-	o	-	-	-	-
	Hernández et al. (2003)	-	-	^	-	-	-	X
	Costa et al. (2012)	-	X	X	-	-	-	-
	Tromm et al. (1992)	X	-	-	-	X	-	-
	Nomura et al. (1986)	-	X	-	-	-	-	-
	Koch et al. (2001)	-	-	-	X	-	-	-
	Bouvier et al. (2006)	-	-	-	X	-	X	-
	Furlong et al. (2005)	-	-	X	-	-	-	-
	Grandjean and Landrigan (2014)	-	-	-	o	o	-	-
	Lopez-Granero et al. (2014)	X	-	-	-	X	-	-
	Marsillach et al. (2011)	-	o	-	-	-	-	-
	Rohlman et al. (2011)	o	o	-	-	o	-	o
	Sanchez-Santed et al. (2004)	X	-	-	-	X	-	-

X = Significant (p<0.05) in bivariate analysis; XX= Significant (p<0.05) in multivariate analysis; o = Factor was not statistically examined;
^ = Factor was examined, no significant association; ~ = Factor was examined, no information regarding association test; - = Factor was not investigated

6. Clinical Manifestations of OP Toxicity

ACh is a neurotransmitter that acts at two main receptors, namely, nicotinic and muscarinic. There are three types of clinical manifestations of OP toxicity: acute cholinergic syndrome, intermediate syndrome, and organophosphate-induced delayed neuropathy (OPIDN). Marrs (2001) indicates that acute cholinergic syndrome and the intermediate syndrome are the result of inhibition of ChE, whereas OPIDN is associated with the inhibition of NTE.

6.1. Acute Cholinergic Syndrome

Acute cholinergic syndrome occurs due to high level exposure to OP compounds. It occurs soon after exposure to OP and lasts for several days. Acute cholinergic symptoms and signs of OP poisoning, including gastrointestinal upset, urination, miosis, bronchospasm, sweating, lacrimation, bradycardia, fasciculations, muscle weakness, hypertension, Central Nervous System (CNS) depression or coma occur as the result of ChE inhibition (Eddleston, 2013; Jaga & Dharmani, 2003). A failure of ChE hydrolysis results excessive amount of ACh (Karalliedde & Henry, 2001; Marrs, 2001). Cholinergic receptors are nicotinic, found at autonomic ganglia and the neuromuscular junction, and muscarinic, found in parasympathetic effector organs (Marrs, 2001). Nicotinic and muscarinic are two types of cholinergic receptors that have a different anatomical locations, different functions, and different clinical symptoms (Figure 1) (Heide, 2007).

The percentage of normal RBC-ChE or PChE activity required to produce acute clinical manifestation is as follows: normal if the activity is greater than or equal to 75%, mild inhibition if the activity ranges from 30% to 74%, moderate inhibition if the activity ranges from 10% to 29%), and severe inhibition if the activity is <10% (Rajapakse et al., 2011).

Heide (2007) grouped signs of OP poisoning based on types of cholinergic receptors (Table 2). An anatomical location of nicotinic and muscarinic receptor targets associated with sign and symptoms of ChE inhibitors are presented in Figure 1.

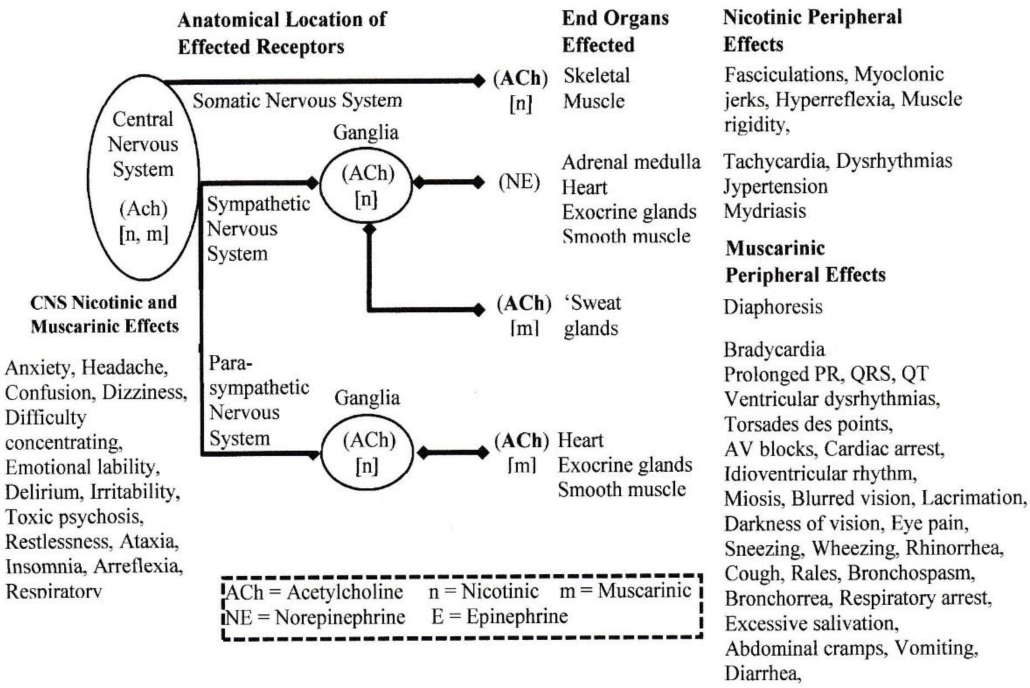


Figure 1: Nicotinic and muscarinic effects of cholinesterase inhibitors. Adapted from Heide (2007).

6.2. Intermediate Syndrome (IMS)

Intermediate syndrome (IMS) develops 1-4 days after acute poisoning and lasts for 18 days. The term "intermediate syndrome" was introduced by Senanayake and Karalliedde in 1987. It appears between acute cholinergic syndrome and OP-induced delayed polyneuropathy (OPIDN) (Bleecker, 2001; Bleecker et al., 1993; Senanayake & Karalliedde, 1987).

IMS (known as the nicotinic syndrome) occurs when the synaptic ChE is inhibited at least 80% after acute exposure (Azazh, 2011). IMS only occurs in the event of severe poisoning (Azazh, 2011). Some clinical manifestations of IMS are weakness of the respiratory system, neck, and proximal limb muscles that occur approximately 16 to 120 h after exposure and 7 to 75 h after the onset of acute pesticide poisoning symptoms (Costa, 2008; National Academies Press, 2003). However, ChE inhibition does not directly influence the occurrence of IMS. Muscle weakness may be affected by prolonging cholinergic stimulation (Costa, 2008).

Table 2: Signs of OP poisoning based on types of cholinergic receptors (Heide, 2007).

Type of Receptor	Location	Signs	Onset
Nicotinic	a) Skeletal neuromuscular junction b) Sympathetic and parasympathetic nervous system c) Autonomic ganglia d) Central nervous system	Mydriasis (pupillary dilation), tachycardia, hypertension, weakness, fasciculations	Soon after OP exposure
Muscarinic	a) Parasympathetic nervous system: • Cardiac conduction system • Exocrine glands • Smooth muscles b) Sympathetic nervous system • Sweat glands c) Central nervous system	Salivation, urination, lacrimation, defecation/diaphoresis, gastrointestinal pain, emesis, miosis (pupillary constriction), bronchospasm, anxiety, emotional lability, hallucinations, restlessness, confusion, depression, headache, respiratory-depression, coma	Slower than nicotinic receptor

A prospective study conducted by Bleecker et al. (1993) indicated that in a 19-patient cohort group, eight patients suffered from IMS, and six of them had relapsing cholinergic signs like lacrimation, increased bronchial and salivary secretion, diarrhoea, sweating, bradycardia, and fasciculation, that superimposed on IMS.

Wananukul et al. (2005), in a 2005 study in Thailand of two cases of OP poisoning in which IMS developed, reported that in the first case, weakness of the neck, proximal limb muscles, and respiratory system developed on the 3rd day after ingestion, and the patient recovered 11 days after the poisoning. The second case developed bulbar palsy, proximal muscle and respiratory weakness 3 days after ingestion.

6.3. OPIDN

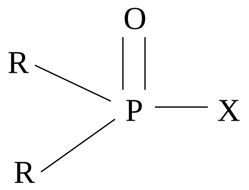
OPIDN starts 1 or 2 weeks after poisoning and is, at least to some extent, persistent. OPIDN is one of the types of toxicity due to some OP insecticides compounds like tri-o-cresyl phosphate, o-ethyl-o-4-nitrophenyl phenylphosphonothionate and o-(4-bromo-2,5-dichlorophenyl)-o-methyl phenylphosphonothionate and can happen in many species including humans. Some clinical manifestations of OPIDN are numbness, weakness of the arms and legs followed by progressive and irreversible ataxia or paralysis which develops 2-3 weeks to months after acute exposure to OP insecticides (Jamal, 1997; Moser et al., 2008; National Academies Press, 2003; Yang & Deng, 2007). It occurs when neuropathy target esterase (NTE) is inhibited in lymphocytes by neuropathic OPs within hours of exposure (Makhaeva et al., 2007). Glynn (2000) defined NTE as '*an integral membrane protein in vertebrate neurons*'. Whole blood NTE is the ideal biomarker to detect OP compounds until 96 h after exposure, in relation with the occurrence of OPIDN (Makhaeva et al., 2007). OPIDN occurs when NTE is inhibited by at least 55%-70% following an acute dose of organophosphate and no

<45% inhibition after repeated exposure (U.S. Environmental Protection Agency, 1998).

There is no association between inhibition of NTE and inhibition of ChE. Not all commercial insecticides (i.e. chlorpyrifos and dichlorvos) lead to OPIDN, unless they are ingested near lethal doses (National Academies Press, 2003).

7. Structures of OP Inhibiting Cholinesterases

The typical general structure of OP inhibiting cholinesterases is as follows (Marrs, 2001):

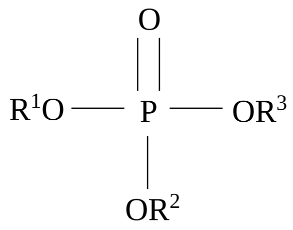


Where P is a central phosphorus, R groups are similar or dissimilar alkoxy groups, O is Oxygen, and X is known as the leaving group.

Specifically, main groups of OP inhibiting cholinesterases are as follows (Marrs, 2001):

a. Phosphate

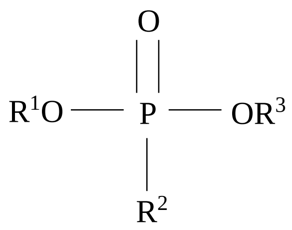
General structure:



Examples: Dichlorvos[a], Chlorfenvinphos[a], Heptenophos[a], and Tri-o-cresyl phosphate[b]

b. Phosphonate

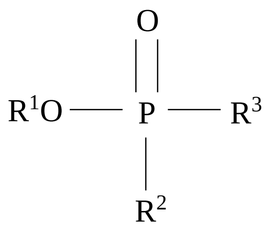
General structure:



Examples: Trichlorfon[a]/Metrifonate[c], and Glyphosate[d]

c. Phosphinate

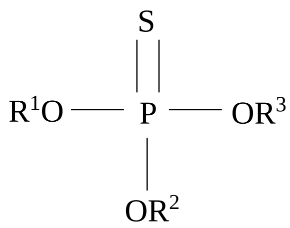
General structure:



Example: Glufosinate[d]

d. Phosphorothioate = S type

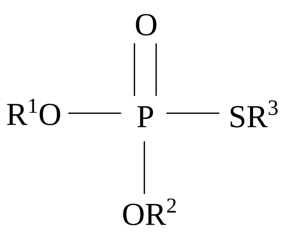
General structure:



Examples: Diazinon[a], Parathion[a], Bromophos[a], Pyrazophos[e], and Fenitrothion[a]

e. Phosphorothioate = S-substituted

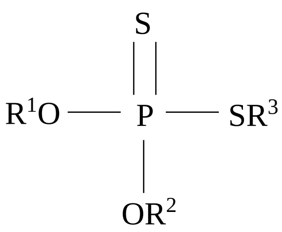
General structure:



Example: Demeton-S-methyl VG[f]

f. Phosphorodithioate

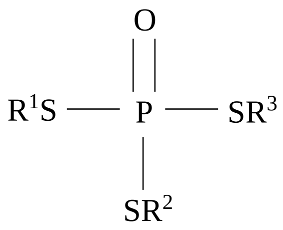
General structure:



Examples: Malathion[a], Dimethoate[a], and Disulfoton[a]

g. Phosphorotrithioate

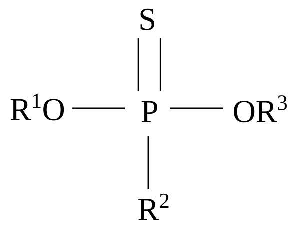
General structure:



Example: S,S,S–Tributyl Phosphorotrithioate (DEF)[g]

h. Phosphonothioate = S type

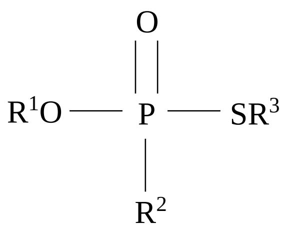
General structure:



Examples: Leptophos[a] and EPN[a]

i. Phosphonothioate = S-substituted

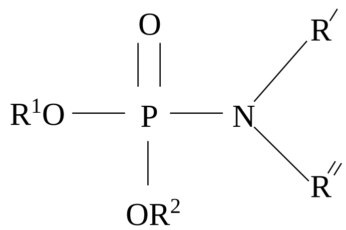
General structure:



Example: VX[f]

j. Phosphoramidate

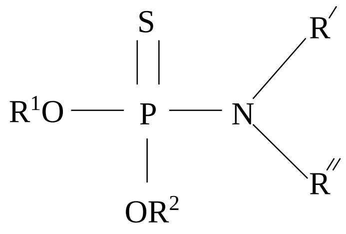
General structure:



Examples: Fenamiphos[a] and Tabun[f]

k. Phosphorothioamidate = S type

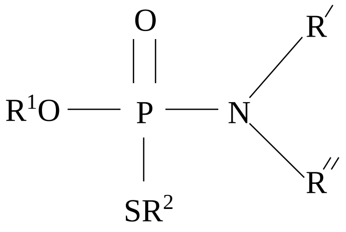
General structure:



Examples: Isofenphos[a] and Propetamphos[a]

l. Phosphorothioamidate = S-substituted

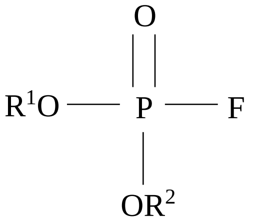
General structure:



Example: Methamidophos[a]

m. Phosphorofluoridate

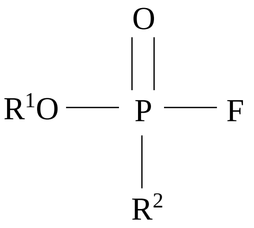
General structure:



Example: DFP[h]

n. Phosphonofluoridate

General structure:



Examples: Sarin[f], Soman[f], and GE[f]

Remarks:

a = Insecticide; b = Industrial chemical; c = INN name used as human pharmaceutical; d = Herbicide; e = Fungicide; f = Chemical warfare agent; g = Defoliant; h = Laboratory chemical

Note: trichlorfon and metrifonate are the same substance.

8. Epidemiological Studies

Studies in around the world have demonstrated that pesticide poisoning is a major public health problem. Some studies have reported the occurrence of acute poisonings from mild to severe poisonings due to OP exposure.

Table 3 presents 17 selected studies of OP-pesticide poisoning among agricultural workers in developing (Afriyanto, 2008; Dasgupta et al., 2007; Faria et al., 2014; He, 1996; Jeyaratnam, 1990; Kir et al., 2013; Kishi et al., 1995; Murali et al., 2009; Peshin et al., 2014; Rajashekhara et al., 2013; Rustia et al., 2010; Zhang et al., 2011) and developed countries (Beseler & Stallones, 2008; Das et al., 2002; Jeyaratnam, 1990; Lee et al., 2011; Zilker, 1996). The basis for the selection of 17 studies was as follows: 1) These studies were conducted in developing countries that might have similar conditions to Indonesia as a developing country and as one of the research sites of my thesis project; 2) These studies investigated agricultural workers; 3) These studies specifically investigated organophosphate pesticide (OP) compounds and OP exposure; 4) These studies investigated poisoning cases due to OP exposure; and 5) These studies presented information of prevalence or incidence of adverse health effects (OP poisoning cases, sign and symptoms, and death) due to OP exposure. The main approaches used in the studies in developing countries were cross-sectional (5 of 12; 41.7%) whereas the studies in developed countries used literature approaches (2 of 5; 40.0%). Most of the studies shown in Table 3 indicate that proportion of OP-pesticide poisoning in developing countries was higher than in developed countries. In developing countries, OP insecticides, are mainly applied in agriculture and are reported to produce adverse health effects (Swaminathan & Widdop, 2001). OP pesticides used in farming worldwide also lead to an increase in morbidity and mortality rates around the world, largely due to an increase in acute pesticide poisoning cases (Jeyaratnam, 1990). The deaths were from occupational OP poisonings. Even though data of worldwide impacts are limited, number of deaths due to pesticide poisoning in 2002 was estimated to be approximately 186,000 and 4,420,000 disability-adjusted life years (Prüss-Üstün & Corvalán, 2006). In developing countries, the number of deaths caused by pesticide poisoning is higher than the mortality caused by infectious diseases (Eddleston et al., 2002).

A prospective cohort study by Eddleston et al. (2005) among 802 patients with chlorpyrifos, dimethoate, or fenthion self-poisoning admitted to three Sri Lankan hospitals from March 31, 2002 to May 25, 2004 demonstrated that the proportion dying due to dimethoate (61 of 264, 23·1%, odds ratio [OR] 3·5, 95% CI 2·2–5·4) was significantly higher than the proportion dying due to fenthion (16 of 99, 16·2%, OR 2·2, 1·2–4·2) or chlorpyrifos (35 of 439, 8·0%).

More recently, a prospective study conducted from September 2008 to January 2010 by Senarathna et al. (2012) in all hospitals with inpatient facilities in Anuradhapura district of North Central Province of Sri Lanka showed that as many as 41% or 1572 of 3813 adult poisoned patients (above 12 years of age) were due to pesticides and approximately 33% of these pesticide poisoning cases were due to OP compounds (chlorpyrifos, dimethoate, malathion, profenophos, other and unknown OP).

In addition to acute poisonings, OP pesticides also cause chronic diseases like cancer, birth defects and developmental toxicity, reproductive disorders, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), diabetes, cardiovascular diseases, chronic nephropathies, chronic respiratory disease, etc. (Mostafalou & Abdollahi, 2013). In developed countries, a cohort study from 1993 to 1997 conducted by Kamel et al. (2007) in the U.S. showed that prevalent and incident cases of Parkinson's Disease associated with pesticides exposure respectively were 83 of 79,557 (0.10%) at enrolment and 78 of 55,931 (0.14%) at follow-up. Another prospective cohort among 57,284 U.S certified/licensed pesticide applicators and 32,333 spouses of private applicators revealed that 300 incident lung cancers linked with OP-pesticide exposure (chlorpyrifos and diazinon) had been observed since enrolment in 1993 until December 2001 (Alavanja et al., 2004). In developing countries, Soetadji et al. (2015) investigated aortic (Ao) elasticity in Indonesia and showed that children living in an OP-exposure area had higher Ao-stiffness index (96%). Another study conducted by Suhartono et al. (2012) among 44 women at childbearing age (WCA) as a case group and 44 WCA as a control group living in agricultural areas indicated that proportion of hypothyroidism due to OP exposure among the case group was 43.2% (19 of 44), whereas the proportion of hypothyroidism among the control group was only 20.0%.

It appears from this review of the literature that, in general, developed countries are more concerned with long term effects of low exposures to insecticides, whereas developing countries remain more focused on acute effects and high level exposures. This may reflect the availability and use of these agents in different countries and may also be a consequence of the awareness of chemical risks and the presence of environmental and consumer lobby groups in developed countries. Further research is necessary to establish the contribution of these factors to pesticide exposures.

Table 3: Available* epidemiological studies on OP pesticides poisoning among agricultural workers in developed and developing countries.

	References	Type of study	Population	Key Findings
Developing countries	Zhang et al. (2011)	Cross-Sectional	910 pesticide applicators from two villages in southern China.	A total of about 80 people or 8.8% of the 910 pesticide applicators suffered from an acute related-work pesticide poisoning. Mostly poisoning cases were due to insecticides exposure (92.5%).
	Kishi et al. (1995)	Prospective cohort	204 farmworkers in Tegal and Brebes Regency, Central Java, Indonesia.	Twenty-one percent of OP pesticide sprayers had at least four symptoms, such as neurobehavioral, gastrointestinal, and respiratory symptoms related to OP pesticides exposure.
	Murali et al. (2009)	Retrospective	All patients hospitalised with acute poisoning during the period of 1990-2004 (15 years) at Nehru hospital of the Postgraduate Institute of Medical Education and Research in Chandigarh.	A total of 2884 patients (1918 men) suffered from acute pesticide poisoning during the period of 1990-2004. OP pesticides were the most common agents (35.1%).
	He (1996)	Retrospective	52,287 cases of acute pesticide poisoning reported from 27 provinces of China in 1993.	6,281 deaths. 17.8% of total cases were due to occupational pesticide poisoning and 78% of total cases of pesticides poisoning were due to OP compounds.
	Rustia et al. (2010)	Cross-sectional	56 pesticide applicators at Campang Village, Gisting Sub District, Tanggamus Regency, Lampung Province, Indonesia	71.4% of 56 participants suffered from mild OP poisoning and 28.6% of 56 farmworkers suffered from moderate OP poisoning.
	Dasgupta et al. (2007)	Survey	190 farmworkers in the Mekong Delta, Vietnam.	All 190 farmworkers had some symptoms after mixing and spraying OP pesticides. These symptoms consisted of skin irritation (66%), headache (61%), dizziness (49%), eye irritation (56%), and shortness of breath (44%).

Available* means "existing" and could be accessed in scientific databases.

(Table 3: Continued)

	References	Type of study	Population	Key Findings
Developing countries	Jeyaratnam (1990)	Literature	Cases of acute pesticide poisonings based on hospital data in Indonesia and Brazil in 1980s	Proportion of total acute poisonings due to pesticides in Indonesia and Brazil in 1980s respectively was 28.0% and 16.0%.
	Faria et al. (2014)	Cross-sectional	2400 tobacco farmers in southern Brazil	Prevalence of minor psychiatric disorders (MPD) due to OP pesticides exposure was 12%. Tobacco farmworkers using OP pesticides had 50% more risk of MPD than those not exposed to OP.
	Peshin et al. (2014)	Retrospective	4929 calls of pesticide poisoning were recorded during 13 year period (1999-2012) by the National Poisons Information Centre, All India Institute of Medical Sciences, New Delhi, India	40.61% of pesticide poisoning cases associated with agricultural pesticides. OP pesticides placed first rank (9.79%) in agricultural pesticides group which caused poisoning.
	Rajashekhara et al. (2013)	Cross-sectional	76 OP poisoned patients who were admitted to Jawaharlal Nehru Medical College	25% of 76 patients were agricultural workers. From the total of the patients, most of them suffered from congested conjunctiva (87%), pin point pupil (83%), lacrimation (80%), vomiting (78%), non-reactive pupil (75%), respiratory distress (60%), and abdominal pain (37%).
	Kir et al. (2013)	Retrospective	10,720 autopsied by Ankara Branch of Council of Forensic Medicine in Turkey	70 cases of 10,720 were attributed to fatal pesticide poisoning. Most of them (63%) was due to OP insecticides.
	Afriyanto (2008)	Cross-sectional	50 chili farmers in Candi Village, Bandungan Sub District, Semarang Regency, Indonesia	13 of 50 participants or 26% suffered from severe OP pesticides poisoning.

(Table 3: Continued)

	References	Type of study	Population	Key Findings
Developed countries	Beseler and Stallones (2008)	Cohort study	872 farmworkers in Colorado, the U.S.	6.0% of 872 respondents on baseline data suffered from pesticide poisoning.
	Das et al. (2002)	Survey	138 farmworkers were selected from 9 cities in the U.S	27.6% of 138 respondents had health problems related to chemicals including pesticides.
	Lee et al. (2011)	Retrospective	2,945 cases from 1998 to 2006 related to agricultural pesticide drift from 11 states in the U.S. Data were obtained from the National Institute for Occupational Safety and Health's Sentinel Event Notification System for Occupational Risk – Pesticides Program and the California Department of Pesticide Regulation.	92% of agricultural workers suffered from low-severity illness. The annual incidence was between 1.39 and 5.32 per million persons over the 9-year period. 45% of cases were due to soil applications with fumigants. Meanwhile, 24% of cases were due to aerial applications.
	Zilker (1996)	Literature	OP poisoning data for Germany between 1975 and 1996.	About 200 OP poisoning cases happen in Germany every year. The poison centre in Munich reported that 482 cases of OP compounds poisoning have occurred from 1975 to 1996. Twenty OP compounds contributed to these cases. The highest incidence was due to parathion (287 cases), oxydemeton-methyl (90 cases), and dimethoate (22 cases). OP compounds were the most commonly used OP in Germany.
	Jeyaratnam (1990)	Literature	Cases of acute pesticide poisonings based on hospital data in U.K, Australia, Canada, and the U.S in 1980s	Proportion of total acute poisonings due to pesticides in U.K, Australia, Canada, and the U.S in 1980s respectively was 5.0%, 3.0%, 2.4%, and 0.8%.

9. Pathways of OP-Pesticide Exposure among Agricultural Workers

The aim of this review is a better understanding how farmworkers are exposed to OP pesticides, and how such exposures can be reduced. The potential risk factors that were frequently included in pathways of OP-pesticide exposure in developing and developed countries were modifiable environmental and behavioural factors. These factors were the most influential factors contributing to good health (Bonita et al., 2006).

The potential environmental risk factors or modifiable determinants for OP-pesticide exposure among agricultural workers based on previous studies in developing (Afriyanto, 2008; Blanco et al., 2005; Issa et al., 2010; Zhang et al., 2011) and developed (Arcury et al., 2014; Early et al., 2006; Flocks et al., 2007; Keller-Olaman, 2005; Lee et al., 2011; Litchfield, 2005; Quackenbush et al., 2006; Ward & Tanner, 2010) countries are presented in Table 4. There are some differences between developing and developed countries regarding environmental factors most commonly relating to OP-pesticide exposures. Generally, hot weather and wind/agricultural pesticide drift appear to be significant factors of higher exposure levels in both country groups. However, other factors, namely poor areas (Zhang et al., 2011) and workplace conditions (Blanco et al., 2005) provided large contributions to OP-pesticide exposure in developing countries. Meanwhile, housing conditions (Arcury et al., 2014; Keller-Olaman, 2005; Ward & Tanner, 2010) and social contextual factors (Keller-Olaman, 2005; Ward & Tanner, 2010) were very significant in developed countries (Table 4).

Table 4: Environmental risk factors and pesticide exposure risk factor studies in developing and developed countries.

Study Sites	References	Environmental related factors							
		Hot weather	Humidity	Housing conditions	A greater number of adults and farmworker in a house	Poor areas	Wind/ Agricultural pesticide drift	Workplace conditions	Social contextual factors
Developing Countries	Issa et al. (2010)	o	-	-	-	-	-	-	o
	Zhang et al. (2011)	-	-	-	-	XX	-	o	o
	Afriyanto (2008)	o	o	-	-	-	-	-	-
	Blanco et al. (2005)	XX	-	-	-	-	-	XX	-
Developed Countries	Quackenbush et al. (2006)	o	-	-	-	-	-	-	-
	Early et al. (2006)	-	-	o	o	-	-	-	-
	Keller-Olaman (2005)	-	-	X	-	-	X	-	X
	Litchfield (2005)	-	-	o	-	-	-	-	-
	Ward and Tanner (2010)	-	-	X	-	-	-	-	X
	Arcury et al. (2014)	-	-	X	-	-	-	-	-
	Lee et al. (2011)	X	-	-	-	-	X	-	-
	Flocks et al. (2007)	o	-	-	-	-	o	o	-

X = Significant (p<0.05) in bivariate analysis; XX= Significant (p<0.05) in multivariate analysis; o = Factor was not statistically examined;
^ = Factor was examined, no significant association; ~ = Factor was examined, no information regarding association test; - = Factor was not investigated

The other group of exposure modifying factors was human behavioural risk factors, which also significantly contributed to OP-pesticide exposure among agricultural workers based on previous studies in developing (Afriyanto, 2008; Blanco et al., 2005; Dosemeci, 2002; Issa et al., 2010; Jintana et al., 2009; Kishi et al., 1995; Lein et al., 2012; Lu, 2007; Mancini et al., 2009; Oluwole & Cheke, 2009; Panuwet et al., 2008; Recena et al., 2006; Ribeiro et al., 2012; Shomar et al., 2014; Zhang et al., 2011) and developed (Arcury et al., 2002; Bradman et al., 2009; Flocks et al., 2007; Hines et al., 2011; Johnstone, 2006; Keller-Olaman, 2005; Stallones, 2002; Strong et al., 2008) countries. The summary of these studies is presented in Table 5. Generally, both developing and developed countries were similar in terms of behavioural factors contributing to OP-pesticide exposure. These factors were as follows: mixing pesticides, spraying pesticides, use of PPE, knowledge, perceptions, washing hands, showering, wearing contaminated clothes, eating, drinking, and smoking. However, each group of countries also had specific behavioural risk factors significantly associated with the exposures. Duration of exposure (Afriyanto, 2008; Kishi et al., 1995; Lu, 2007; Mancini et al., 2009), pesticide safety training (Zhang et al., 2011), frequency of pesticide application (Kishi et al., 1995), spraying against the wind (Afriyanto, 2008), and reuse of pesticide containers for storage (Kishi et al., 1995; Lu, 2007) were very significant factors in developing countries. Riding on equipment (Hines et al., 2011; Stallones, 2002) was reported to be a significant factor in developed countries (Table 5).

Table 5: Behavioural risk factors and pesticide exposure risk factor studies in developing and developed countries.

Study Sites	References	Behavioural related factors: Mixing pesticides	Loading pesticides	Spraying pesticides	Touched sprayed crops	Riding on equipment	Duration of exposure	Use of PPE	Knowledge	Perceptions	Pesticide safety training	Washing hands	Taking a shower	Wearing contaminated clothes	Re-entry into a farm area after pesticide spraying	Eating, Drinking, or Smoking	Illiteracy	Frequency of applying pesticides	Spraying against the wind	Reuse of pesticide containers for storage
Developing Countries	Ribeiro et al. (2012)	o	o	o	-	-	-	o	-	-	o	o	o	-	o	-	-	-	-	-
	Shomar et al. (2014)	o	-	o	-	-	-	o	o	-	-	-	-	-	-	-	-	-	-	-
	Dosemeci (2002)	~	-	~	-	-	-	-	-	-	-	~	-	-	-	-	-	-	-	-
	Lein et al. (2012)	^	-	^	-	-	-	X	-	-	-	-	-	-	-	^	-	-	-	-
	Mancini et al. (2009)	X	o	o	-	-	X	-	-	-	-	-	-	-	-	-	XX	-	-	-
	Panuwet et al. (2008)	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	^	-	-
	Zhang et al. (2011)	-	-	^	-	-	-	XX	-	-	XX	-	^	-	-	XX	-	-	^	-
	Jintana et al. (2009)	X	-	-	-	-	^	X	-	-	-	-	^	^	-	^	-	-	-	-
	Oluwole and Cheke (2009)	o	-	o	-	-	-	o	-	-	o	-	-	o	-	-	o	-	-	-
	Lu (2007)	o	o	o	-	-	X	-	-	-	-	-	-	X	^	-	-	-	-	X
	Afriyanto (2008)	-	-	X	-	-	X	X	X	X	-	X	X	-	-	-	-	-	X	-
	Issa et al. (2010)	^	-	o	-	o	o	-	-	-	-	-	-	-	-	X	-	^	-	-
	Kishi et al. (1995)	o	o	X	-	-	X	X	-	-	-	-	-	X	-	o	-	XX	-	X
	Recena et al. (2006)	-	-	-	-	-	^	^	o	o	-	X	^	^	-	^	-	-	^	o
	Blanco et al. (2005)	-	-	XX	-	-	-	~	-	-	-	~	-	-	-	-	-	-	~	-

X = Significant (p<0.05) in bivariate analysis; XX= Significant (p<0.05) in multivariate analysis; o = Factor was not statistically examined;
^ = Factor was examined, no significant association; ~ = Factor was examined, no information regarding association test; - = Factor was not investigated

(Table 5: Continued)

Study Sites	References	Behavioural related factors																		
		Mixing pesticides	Loading pesticides	Spraying pesticides	Touched sprayed crops	Riding on equipment	Duration of exposure	Use of PPE	Knowledge	Perceptions	Pesticide safety training	Washing hands	Taking a shower	Wearing contaminated clothes	Re-entry into a farm area after pesticide spraying	Eating, Drinking, or Smoking	Illiteracy	Frequency of applying pesticides	Spraying against the wind	Reuse of pesticide containers for storage
Developed Countries	Hines et al. (2011)	X	-	X	-	X	-	XX	-	-	-	-	-	-	-	-	-	-	-	-
	Stallones (2002)	^	^	^	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Strong et al. (2008)	-	-	-	-	-	-	o	-	X	o	o	o	o	-	-	-	-	-	-
	Arcury et al. (2002)	-	-	-	-	-	-	X	X	X	-	X	X	X	-	-	-	-	-	-
	Johnstone (2006)	^	^	-	-	-	^	^	o	-	-	X	-	-	-	^	-	-	-	-
	Bradman et al. (2009)	-	-	-	-	-	X	XX	-	-	-	-	-	o	-	XX	-	-	-	-
	Keller-Olaman (2005)	-	-	-	-	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-
	Flocks et al. (2007)	-	-	-	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-

X = Significant (p<0.05) in bivariate analysis; XX= Significant (p<0.05) in multivariate analysis; o = Factor was not statistically examined;
^ = Factor was examined, no significant association; ~ = Factor was examined, no information regarding association test; - = Factor was not investigated

10.Conclusions

Farmers, market gardeners, pesticide applicators, mixers, loaders, flaggers, and their families have been badly affected by pesticide exposure (Maddy et al., 1986; Maroni et al., 1986; Wilson et al., 2005). Flaggers are persons involved in vehicle washing or cleaning aircraft, pickups, autos, or washing or cleaning equipment exposed to OP pesticides (aerial spray workers) (Maddy et al., 1986). Morbidity and mortality rate due to pesticide exposure have risen dramatically since the first use of pesticides thousands of years ago. The number of studies exploring the impact of pesticide exposure on farmer health is increasing. Evaluation of the existing literature is as follows:

1. Scientific research examining factors contributing to OP-pesticide exposure among farmworkers by comparing developed countries and developing countries remains limited. In general, results indicate that the resulted data were only partially analysed that means previous researchers did not compare developing and developed countries. They only presented data only in a developing country or in a developed country.
2. Few studies have looked at the biological monitoring like levels of cholinesterase in blood and types of metabolites in urine samples, and adverse health effects due to pesticide exposure associated with risk factors. Most studies looked only at risk factors of pesticide exposure without direct biomarker measurements.
3. Environmental and behavioural risk factors like hot weather, wind/agricultural pesticide drift, pesticide mixing, pesticide spraying, use of PPE, knowledge, perceptions, washing hands, showering, the wearing contaminated clothes, eating, drinking, and smoking significantly influenced the increase of OP-pesticide exposure in both groups of countries.

4. Poor areas, workplace conditions, duration of exposure, pesticide safety training, application frequency, spraying against the wind, and reuse of pesticide containers for storage were specific factors available in developing countries which contributed to the increase of OP-pesticide exposure. Meanwhile, specific factors in developed countries were housing conditions, social contextual factors, and riding on equipment.
5. Improved knowledge can improve behaviour, perception, psychosocial factors, and public health. Few studies have been conducted on the effect of improving farmer's knowledge by conducting pesticide safety training associated with biomarkers.
6. Most of the studies conducted in developing countries used cross-sectional design. Meanwhile, a literature approach was very common in developed countries. There was an absence of integrated studies regarding OP exposure, risk factors, and outcome.
7. Finally, improving knowledge about the adverse effect of pesticides and knowledge about self-protection from pesticide exposure has the potential to make a significant impact on improving pesticide handling, storage, and application.

Despite research exploring the risk factors of pesticide exposure and its impact on farmer's health, we still do not know much about the specific pathways that may increase pesticide exposure for farmers in developing and developed countries. Particularly concerning, there is a clear paucity of relevant research in Indonesia and Australia. At this point, we can conclude that well-designed quasi experimental studies are needed to highlight the benefits of providing pesticide safety training for preventing and reducing pesticide exposure among farmworkers.

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Conflict of Interest

The authors declare that they have no conflicts of interest to report.

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Chapter 2. Introduction

Pesticides are natural or synthetic chemicals used to control pests, vectors of human or animal disease, unwanted species of plants or animals causing harm either before or after the harvest (FAO, 2009). Pesticide benefits include improving crop yields and efficiency of food production processes, reducing the cost of food, providing high-quality produce for consumers, maintaining aesthetic quality, protecting human health from disease-carrying organisms, suppressing nuisance-causing pests, and protecting other organisms including endangered species from pests (Damalas, 2009; Sheldon, 2010).

Organophosphates pesticides (OPs) are among the most widely used agricultural chemicals. More than 100 OP compounds have been developed and used in many countries around the world (Kapka-Skrzypczak et al., 2011). Chlorpyrifos, diazinon, and malathion are the most common OP compounds used by farmworkers (Heide, 2007; Weiss et al., 2004; WHO, 2009).

OPs have the capacity to contribute to mortality and morbidity where their use is poorly controlled (Jeyaratnam, 1990). In developing countries, the number of deaths due to pesticide poisoning is more than the number of deaths due to infectious diseases (Eddleston et al., 2002). OP groups are the biggest cause of poisoning (Kusnoputranto, 1995).

Environmental exposure, personal behaviour, and inherited characteristics that increase a possibility of a person developing a disease are considered as risk factors for disease (Porta, 2008). The increase of OP pesticide exposure in both developed and developing countries are influenced by environmental risk factors such as hot weather, wind/agricultural pesticide drift, and behavioural factors such as mixing pesticides, spraying pesticides, use of personal protective equipment (PPE), knowledge, perceptions, washing hands, taking a shower after applying OPs, wearing contaminated clothes, eating, drinking, and smoking during working with OPs (Afriyanto, 2008; Arcury et al., 2002; Blanco et al., 2005; Bradman et al., 2009; Johnstone, 2006; Lee et al., 2011; Lein et al., 2012; Mancini et al., 2009; Panuwet et al., 2008; Ribeiro et al., 2012; Shomar et al., 2014; Zhang et al., 2011). Few studies have been conducted to improve farmers' knowledge by conducting pesticide safety training associated with biomarkers (Suratman et al., 2015, Chapter 1).

To test whether worker exposures and adverse effects associated with pesticides may be attributed, at least in part, to poor knowledge about pesticide toxicity, poor handling and/or training, inappropriate work practices or poor infrastructure at the work site, workers from Indonesia and South Australia were examined. A questionnaire sought to assess the level of knowledge, perception of risks, handling characteristics and equipment used for applying pesticides, as well as whether there were facilities available for reducing exposure (such as handwashing, personal protective equipment etc.). Following a short information educational intervention, in which workers were provided with an information session to improve their knowledge and work practices, the questionnaire was readministered. Changes in responses attributable to the intervention were recorded. Confirmation that farmworkers experience some adverse effect following OP exposure was by collection of a fingerprick blood sample before and following the intervention. Fingerprick blood samples were assessed immediately using a hand held field testing instrument (Test-mate ChE Cholinesterase Test System®) to measure erythrocyte acetylcholinesterase (EAChE) and plasma cholinesterase (PChE) activities. The following chapters of this thesis contain a series of papers exploring risk factors for OP exposure among Indonesian and South Australian migrant farmworkers and the impact of an intervention to reduce exposure. The aims of these chapters are as follows:

Chapter 3

Chapter 3 examines the effectiveness of the intervention on OP-related knowledge and perceptions through conducting a quasi-experimental study. This chapter represents the first intervention targeted particularly at reducing OP exposure among Indonesian and SA migrant farmworkers that has been assessed for behavioural changes and compared with health behaviour theory. The objectives of the interventions were to improve knowledge and perceptions about OP exposure among Indonesian and SA migrant farmworkers and to measure the effectiveness of the interventions using two different methods, namely teaching in a class (power point slide and discussion) for Indonesian farmworkers and individual approach (flipchart and discussion) for SA migrant farmworkers.

Chapter 4

Chapter 4 describes the differences in field practices in handling OPs and the prevalence of OP-related symptoms among Indonesian and South Australian (SA) migrant farmworkers between pre and post educational intervention. This chapter describes some aspects of field practices in handling OPs as follows: activities associated with OP application; methods of OP application; types of PPE usually worn by farmworkers during working with OPs; personal hygiene behaviour by Indonesian and SA migrant farmworkers when working with OPs; types of packaging and active ingredients of OPs products; workplace conditions; and OP-related symptoms.

Chapter 5

Chapter 5 presents the results of a study measuring activity levels of erythrocyte acetylcholinesterase (EAChE) and plasma cholinesterase (PChE) in Indonesian and South Australian (SA) migrant farmworkers to assess exposure to OPs, pre and post educational intervention. This chapter reports OP induced enzymes inhibition measured in blood samples. EAChE and PChE levels were measured using 10μL fingerprick blood samples each with the Test-mate ChE Cholinesterase Test System® field kit.

Chapter 6

Chapter 6 aims to examine whether the interaction between pralidoxime (pyridine-2-aldoxime methochloride) solution in saline leads to changes in PChE activities inhibited by OPs using fresh plasma blood samples in field measurements to estimate percent inhibition of PChE activities due to OP exposure. This chapter reports the results of a true experimental study to measure the utility of pralidoxime reactivation of OP-induced PChE inhibition in fresh fingerprick blood samples as an exposure monitoring assay to be used under field conditions. One 8μL portion of fresh plasma blood sample was mixed with 2μL pralidoxime solution in saline whereas the other portion was mixed with 2μL saline solution. Test-mate ChE Cholinesterase System Test field kit was used to analyse PChE activities.

Chapter 7

Chapter 7 interprets and discusses the overall study outcomes. Results from research chapters are assessed to determine what measures can be taken to improve the health status of farmworkers, and identifies changes that can be implemented to change farmworkers' behaviour.

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Chapter 3. Knowledge and Perceptions of OP exposure

The Effectiveness of an Educational Intervention to Improve Knowledge and Perceptions for Reducing Organophosphate Pesticide Exposure among Indonesian and South Australian Migrant Farmworkers

Suratman[1,2], **Kirstin E Ross**[1], **Kateryna Babina**[1], **John William Edwards**[1]

1. Health and Environment Group, School of the Environment, Faculty of Science and Engineering, Flinders University, Adelaide, SA, Australia.
2. School of Public Health, Faculty of Health Sciences, Jenderal Soedirman University, Kampus Karangwangkal, Purwokerto 53122, Indonesia.

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Group communication; individual communication; organophosphate pesticides exposure; Indonesian farmworkers; South Australian migrant farmworkers.

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Abstract

Background: Farmworkers are at risk of exposure to Organophosphate Pesticides (OPs). Improvements of knowledge and perceptions about organophosphate (OP) exposure may be of benefit for reduction in OP exposure. **Purpose:** The purpose of this study was to examine the effectiveness of an educational intervention to improve knowledge and perceptions for reducing OP exposure among Indonesian and South Australian (SA) migrant Farmworkers. **Methods:** This was a quasi-experimental study. The educational intervention used a method of group communication for 30 Indonesian farmworkers and individual communication for seven SA migrant farmworkers. Knowledge and perceptions about OP exposure were measured pre intervention and 3 months after the intervention. **Results:** Unadjusted intervention effects at follow-up showed statistically significantly improved scores of knowledge (both adverse effects of OPs and self-protection from OP exposure), perceived susceptibility, and perceived barriers among Indonesian farmworkers compared with SA migrant farmworkers. Furthermore, these four significant variables in the unadjusted model and the two other variables (perceived severity and perceived benefits) statistically were significant after being adjusted for level of education and years working as a farmworker. In contrast, knowledge about adverse effects of OPs was the only variable that was statistically significantly improved among SA migrant farmworkers. The results of this study suggest educational interventions using a method of group communication could be more effective than using individual intervention. **Conclusion:** These improvements provide starting points to change health behaviour of farmworkers, particularly to reduce OP exposure, both at the workplace and at home.

1. Background

Organophosphorus pesticides (OPs) are highly toxic and exposure to OPs contributes to mortality and morbidity when their use is poorly controlled (Jeyaratnam, 1990). Farmworkers are at risk of expose to OPs. There is overwhelming epidemiological evidence that organophosphate (OP) use poses significant health risks if undertaken without safe handling practices. Studies in developing countries (Dasgupta et al., 2007; Faria et al., 2014; Kishi et al., 1995; Rustia et al., 2010; Zhang et al., 2011) and developed countries (Beseler & Stallones, 2008; Das et al., 2002; Lee et al., 2011) have demonstrated acute and chronic effects due to OP exposure.

A study by He (1996) in the People's Republic of China showed that as many as 18% of 6,281 deaths (deaths due to acute pesticide poisoning) were due to occupational pesticide poisoning and 78% of these cases of pesticide poisoning were due to OP compounds in the year 1993. In addition, a study by Dasgupta et al. (2007) in Vietnam in 10 districts of 5 provinces in the Mekong Delta (An Phu and Chau Thanh (An Giang province), Thot Not and Vi Thanh (Can Tho province), Tan Thanh and Thu Thua (Long An province), Cai Lay and Cho Gao (Tien Giang province), and Tra Cu and Tieu Can (Tra Vinh province) in the Mekong Delta demonstrated that all 190 participant farmworkers had some ill health symptoms after mixing and spraying OPs, including other agri-chemicals or co-formulants such as solvents and extenders. These symptoms consisted of skin irritation (66%), headache (61%), dizziness (49%), eye irritation (56%), and shortness of breath (44%).

A wide range of measures exist for reducing health risks from OP exposure. Suratman et al. (2015, Chapter 1) demonstrated that farmworkers' knowledge and perceptions were two of the factors significantly related to the increase of OP exposure and OP poisoning both in developing and developed countries. In

Indonesia, Afriyanto demonstrated that occurrence of OP poisoning among chilli sprayers was significantly influenced by knowledge and perceptions (Afriyanto, 2008). On the other hand, Johnstone et al studied OP exposure in Australian agricultural workers and found that > 75% of farmworkers had a good knowledge about safe handling practices (Johnstone et al., 2007).

OP exposure is a major occupational health concern particularly in Indonesia (Afriyanto, 2008; Kishi et al., 1995; Rustia et al., 2010). A study by Kishi et al. (1995) reported that 21% of OP pesticide sprayers had at least three or more symptoms, such as neurobehavioral, gastrointestinal, and respiratory symptoms related to OP exposure. Protective clothing, such as long-sleeved shirts, knee-high or long pants, and coveralls, and personal protective equipment (PPE), such as chemical-resistant gloves, eye protectors, head gear, and footgear are required during handling and applying OPs. They can reduce dermal contact and inhalation exposures (Fenske & Edgar W. Day, 2005). However, improvement of farmworkers' knowledge and perceptions is required for them to adopt these protective health behaviours such as the use of PPE. According to Rogers (1983), improvement in knowledge is the first stage to adopting new ideas, playing an important role in changing farmworkers' behaviour, particularly in protecting themselves from OP exposure. A study by Arcury et al. (2002) with 293 participant farmworkers in North Carolina, USA, demonstrated that knowledge of pesticide exposure had a significant relationship with perceived risk. In addition, safety knowledge was strongly related to perceived control. Another study by Zyoud et al. (2010) with 381 participant farmworkers in Palestine showed that pesticide knowledge was significantly associated with work practices in handling pesticides in the field.

Low knowledge about adverse effects (AEs), perceived low severity of OP exposure and perceived insusceptibility to OP toxicity were risk factors of inappropriate handling of OP compounds in Indonesia (Afriyanto, 2008). In contrast, fruit and vegetable farmworkers in Australia generally have a good level of knowledge and perceptions of OP exposure (Johnstone et al., 2007). Educational interventions using a group communication and one-on-one approach, and the comparisons of knowledge and perceptions about OP exposure between farmworkers in Indonesia and migrant farmworkers in Australia had not been investigated previously (Suratman et al., 2015, Chapter 1).

These reports suggest that improvements of knowledge and perceptions about OP exposure among Indonesian farmworkers and South Australian (SA) migrant farmworkers may be of benefit for reduction in OP exposure. The objective of the interventions in this study was to improve knowledge and perceptions about OP exposure among Indonesian and SA migrant farmworkers and to measure the effectiveness of provided interventions using two different methods, namely teaching in a class (PowerPoint slide and discussion) for Indonesian farmworkers and an individual approach (flipchart and discussion) for SA migrant farmworkers. In this paper we present the effects of both interventions on OPs-related knowledge and perceptions. This was measured by conducting a quasi-experimental study. This paper represents the first intervention targeted particularly at reducing OP exposure among Indonesian and SA migrant farmworkers that has been assessed for behavioural changes and compared with Health Belief Model (HBM) theory.

2. Materials and Methods

2.1. Study Population

This quasi-experimental study was conducted in two research sites, Dukuhlo Village in Brebes Regency, Central Java province, Indonesia and in the suburb of Virginia, Adelaide, South Australia, Australia. The choice of these distinct populations was due to a clear paucity of relevant research comparing knowledge and perceptions of OP exposure among farmworkers working and living in Indonesia as a developing country and in Australia as a developed country (Suratman et al., 2015, Chapter 1). Inclusion criteria were: 1) male; and 2) had to be employed in farm work within the past 3 months. These criteria were based on the following: 1) The majority of farmworkers in 2010-2011 in Australia (139,500 or 72%) (Australian Bureau of Statistics, 2012) and in 2013 in Indonesia (24.36 million or 77%) (Indonesian Bureau of Statistics, 2013) were male; 2) Engaging in farm work within the past 3 months reflected recent likelihood of being exposed to OPs. In addition, complete recovery of plasma cholinesterase (PChE) as a biomarker of exposure to OPs and erythrocyte cholinesterase as a biomarker of toxicity is 50 days and 82 days, respectively (Mason, 2000).

Thirty Indonesian farmworkers were given the educational intervention material through group presentations, whereas seven SA migrant farmworkers were given the intervention material during a one on one with the researcher. The ethnicity of SA migrant farmworkers was Vietnamese. Migrant farmworkers were born in Vietnam (Vietnamese ethnicity) and moved from Vietnam to South Australia to do farm works. They were identified by asking their places and dates of birth when started interviewing them using a questionnaire in the baseline data collection (the pre-intervention). Previous studies in developed countries have indicated that migrant farmworkers face a greater risk of illnesses and death due to pesticides exposure than the indigenous farming community (Arcury & Quandt, 2003; Ciesielski et al., 1994; Levine, 2007; Reidy et al., 1992). This study was conducted from May to June 2014 in Australia and from July to August 2014 in Indonesia for the baseline study (pre intervention). Follow-up studies (post intervention) were conducted from September to October 2014 in Australia and from November to

December 2014 in Indonesia. The questions on personal characteristics were administered at baseline, before the intervention. The questions on knowledge and perceptions were administered at baseline and at 3 months after the intervention. Ethics approvals were obtained from Southern Adelaide Clinical Human Research Ethics Committee (SACHREC) with approval number: 319.13, and from the Commission on Health Research Ethics, Faculty of Public Health, Diponegoro University, Semarang, Indonesia with approval number: 183/EC/FKM/2013. After participants signed the informed consent, they were then interviewed.

2.2. Sample Size Estimation

The required sample size was calculated based on previous studies (EQM Research, 2011; Miranda-Contreras et al., 2013) using STATA IC/12.1 software (StataCorp LP, College Station, TX, USA). This program is used to determine the minimum number of participants needed for each research site, with power of the test =90%, level of significance =0.05, mean ± standard deviation (SD) =1.5 ± 0.3 U/mL of PChE level (also known as butyrylcholinesterase) as a biomarker of exposure to OPs (30%-74% of normal) (Miranda-Contreras et al., 2013), and mean ± SD =2.0 ± 0.4 U/mL of normal PChE level in a population (EQM Research, 2011). Sample size required for this study for each group was 20. In Indonesia, 30 of 52 Indonesian farmworkers working and living at the Dukuhlo Village were randomly selected to accommodate missing data and possible dropout using a random number table generated by C-Survey v2.0 free software (Muhammad N Farid and Ralph R Frerichs, Los Angeles, USA). On the other hand, due to many difficulties in recruiting research participants in Australia, a snowball sampling method was used, which involved asking research participants to nominate another farmworker. This resulted in seven SA migrant farmworkers working and living in Virginia, South Australia, being included in this study.

2.3. Research Questionnaire Instrument

HBM theory was used to explain behavioural factors (knowledge and perceptions) as a basis for interventions. According to the HBM, there are four factors directly associated with individual behaviours. These factors consist of perceived susceptibility, perceived severity, perceived benefits, and perceived barriers which are modified by other variables such as culture, education level, age, sex, ethnicity, past experience, knowledge, and cues to action (Champion & Skinner, 2008; Stretcher et al., 1997). Perceived susceptibility is a powerful perception, which leads farmworkers to adopt healthier behaviours. Farmworkers must perceive their susceptibility to risk before they will take action.

The questionnaire was written in English and was translated into Indonesian language. The questionnaire data collection both in Indonesia and in Australia was conducted by the first author in face to face interviews (interviewer-administered questionnaire). The author clarified and explained misunderstood questions. This did not lead the interviewees. In Indonesia, the first author, of native Indonesian ancestry, used Indonesian language to ask all questions. In Australia, the first author used English to collect data from SA migrant farmworkers. We did not assess the level of their English proficiency before we asked questions. However, more than half of the research participants (57%) could speak English well. When we interviewed research participants who could not speak English fluently, we asked for help from someone, such as a family member who was fluent in English to translate, in order to avoid misunderstanding in answering questions. Original questions were developed for knowledge about AEs of OPs (Appendix A), knowledge about self-protection from OP exposure (Appendix B), and perceptions about OP exposure (perceived susceptibility, perceived severity, perceived benefits, perceived barriers, and cues to action) (Appendix C). The questionnaire consisted of: 1) personal characteristics - age, years working as a farmworker, and level of education,; 2) knowledge about AEs of OPs as assessed by 12 close-ended questions; 3) knowledge about self-protection from OP exposure as assessed by ten close-ended questions; 4) perceptions about OP exposure as assessed by 20 close-ended questions. These 20 questions about perceptions encompassed perceived susceptibility (six questions), perceived severity (four questions), perceived benefits (two questions), perceived barriers (four questions), and cues to action (four questions).

For true/false questions, if the question was answered correctly, the score was 2. If the respondents answered “don’t know”, the score for that question was 1, and if they answered incorrectly, the score was 0. Total possible score of knowledge about AEs of OPs ranged from 0 to 24 and total possible score of knowledge about self-protection from OP exposure ranged from 0 to 20.

The questions of perceptions had five response options, namely ‘strongly disagree’, ‘disagree’, ‘neutral’, ‘agree’, and ‘strongly agree’ using Likert scale ranging from 5 for positive perception answer to 1 for negative perception answer.

Positive statement questions contained a statement which may lead farmworkers to practice healthy behaviour in reducing OP exposure (e.g. C11. “Use of PPE will protect the body from AEs of pesticide exposure”, Appendix C). On the other hand, negative statement questions were aimed at a belief, which may inhibit farmworkers to practice healthy behaviour in reducing OP exposure (e.g. C9. “The effect of pesticide on the body is easily cured”, Appendix C). Total possible perception scores ranged from 6 to 30 for perceived susceptibility, 4 to 20 for perceived severity, 2 to 10 for perceived benefits, 4 to 20 for perceived barriers, and

4 to 20 for cues to action. The knowledge questions and perceptions questions are presented in the Appendices A-C.

The questionnaire was validated with pilot testing for clarity and reliability on 12 non-occupationally exposed persons, by the first author. Pearson's product moment correlation (*r*) and Cronbach's alpha tests were calculated to assess construct validity and internal consistency, respectively. Construct validity measured by the correlation between a score from an individual question and a total score of all questions showed the *r* for individual knowledge and perceptions questions was >0.50 ($p<0.05$). Meanwhile, Cronbach's alpha demonstrated good reliability, with Cronbach's alpha for knowledge about AEs of OPs, knowledge about self-protection from OP exposure, and perceptions about OP exposure 0.72, 0.71, and 0.73 respectively (where 0 is unreliable and 1 is very reliable). Tests of validity and reliability for a translated questionnaire (Indonesian language) were not conducted. The first author translated the questionnaire ensuring the meaning of each translated question written in Indonesian was the same as in the English questionnaire.

The intervention program in each group lasted for 1 hour based primarily on the HBM theory to improve knowledge and perceptions of OP exposure. The provided information covered the following: 1) definition of pesticides; 2) groups of pesticides; 3) pathways of OP exposure at workplace and at home; 4) adverse health effects of OPs; 5) signs and symptoms of acute and chronic effects due to OP exposure; 6) self-protection from OP exposure at workplace; 7) self-protection from OP exposure at home; 8) PPE; and 9) first aid when exposed to OP exposure.

The interventions used two modes of educational delivery: a PowerPoint presentation was used for Indonesian farmworkers and flipchart was used for SA migrant farmworkers with the same content. Different methods of educational interventions were used to accommodate the local conditions. In Indonesia, the intervention using PowerPoint presentation was suitable for the Indonesian community because the research participants lived in the same village. Thirty Indonesian farmworkers were divided into two groups (the first group consisted of 20 farmworkers and the second group consisted of ten farmworkers). This was to ensure the audiences were not too large (no more than 20 persons per group intervention). The participants were gathered at a village hall on separate days for each group. The information was conveyed by the first author, using Indonesian language and was followed by a discussion (Figure 1). Meanwhile, SA migrant farmworkers were provided intervention in English language using flipchart followed by a discussion (Figure 2). For SA migrant farmworkers, the intervention was delivered individually, which was a suitable method for farmworkers who did not live in the same place and the researcher needed to present the material at their workplace (the farm) by prior appointment to accommodate the participants' work schedules.

Figure 1: Providing intervention used a group communication method at Dukuhlo Village, Brebes Regency, Indonesia.

Figure 2: Providing intervention used a one-on-one method at Suburb of Virginia, North Adelaide, SA, Australia.

2.4. Data Analysis

Descriptive statistics were used to describe mean, SDs, frequencies, percentages for personal characteristics, and for knowledge and perceptions scores. Continuous data were tested for normal distribution using the Shapiro-Wilk test (Elliot & Woodward, 2007; Razali & Wah, 2011). Baseline differences in knowledge and perceptions between Indonesian and SA migrant farmworkers were tested by either unpaired *t*-test or Mann-Whitney *U* test.

At follow-up (post-intervention), the magnitude of the intervention effect was the difference between Indonesian and SA migrant farmworkers in the change of mean score from pre-intervention to post-intervention. The outcome measure was the difference in the magnitude of intervention effect between before and after intervention and between the study groups. It was assessed as the change in the mean scores of knowledge and perceptions about OP exposure from the baseline data (pre-intervention) to follow-up (post-intervention).

Linear mixed models were constructed to test the statistical significance of intervention effects on knowledge and perception scores measured 3 months after the intervention (follow-up time). Unadjusted fixed-effects models were used to assess the main effects of intervention and follow-up time, and an intervention-time interaction term for follow-up time. The first model consisted of time as the repeated measure, the study participant as the individual subject, and an unstructured covariance type. Level of statistical significance (*p-value*) was set at $\alpha = 0.05$.

In a second model, used to control confounding variables, interventions effects were adjusted for level of education and years working as a farmworker. These significantly differed between Indonesian and SA migrant farmworkers ($p<0.05$), reported in pre intervention. These two variables significantly influenced knowledge and perceptions of farmworkers (Boonyakawee et al., 2013; Parveen & Nakagoshi, 2001). Intervention effects are therefore presented as absolute magnitudes and percentages of baseline mean scores. Statistical analyses were performed using the statistical package SPSS version 17 (SPSS Inc., Chicago, IL, USA).

3. Results

Variables of personal characteristics are summarised and compared between Indonesian and SA migrant farmworkers in Table 1. Years working as a farmworker was statistically significantly higher in Indonesian farmworkers than SA migrant farmworkers. Meanwhile, level of education was statistically significantly higher in SA migrant farmworkers than Indonesian farmworkers. Thus, intervention effects on knowledge and perceptions were adjusted for these characteristics. Age was not significantly different between the two study groups ($p>0.05$), so no adjustment was made for its variable.

Table 1: Baseline characteristics compared between Indonesian and SA migrant farmworkers.

Characteristics	Indonesian farmworkers (n=30)		SA migrant farmworkers (n=7)		*p-value**
	Mean	**SD**	**Mean**	**SD**	
Continuous variables:					
Age (year)	54.1	7.2	50.9	13.0	0.364
Years working as a farmworker	31.3	9.1	16.7	11.6	0.001
Categorical variable:					
	n	**%**	**n**	**%**	***p-value***
Level of education:					0.000
Never	4	13.3	0	0.0	
Elementary School	20	66.8	0	0.0	
Junior High School	4	13.3	0	0.0	
Senior High School	1	3.3	2	28.6	
Diploma (D1/D2/D3)	1	3.3	3	42.8	
University	0	0.0	2	28.6	

Note: *By unpaired t test; **by chi-square test
Abbreviation: SA, South Australian; SD, standard deviation

Unadjusted intervention effects at follow-up are shown in Table 2. The intervention was related to substantial and statistically significant improvement in scores of knowledge about AEs of OPs, knowledge about self-protection from OP exposure, perceived susceptibility, and perceived barriers at follow-up time ($p \leq 0.05$). Meanwhile, scores of perceived severity, perceived benefits, and cues to action did not statistically improve at follow-up time ($p > 0.05$).

For example, from baseline to follow-up, scores of knowledge about AEs of OPs increased by 3 points more in Indonesian farmworkers than SA migrant farmworkers. This represented an intervention-related improvement of 21.9% of the baseline mean score of knowledge about AEs of OPs.

Table 2: Absolute magnitudes of unadjusted intervention effects on knowledge score and perceptions score, and intervention effects as percentages of baseline and follow-up scores.

Variables	Overall mean at baseline	Intervention effects (unadjusted)		
		Follow-up		
		Absolute magnitude (95% CI)	*p-value*	As % of baseline mean
Score of knowledge about adverse effects of OPs	13.7	3.0 (1.6-4.4)	< 0.001	21.9
Score of knowledge about self-protection from OP exposure	14.8	1.3 (0.1-2.5)	0.040	8.8
Score of perceived susceptibility	20.9	1.7 (0.5-2.9)	0.007	8.1
Score of perceived severity	9.9	0.5 (-0.1-1.1)	0.115	5.1
Score of perceived benefits	8.4	0.5 (-0.4-1.3)	0.271	5.9
Score of perceived barriers	9.9	0.7 (0.2-1.3)	0.012	7.1
Score of cues to action	13.9	0.2 (-0.3-0.8)	0.425	1.4

Abbreviations: CI, confidence interval; OPs, organophosphate pesticides; OP, organophosphate.
Note: *p-value* refers to the difference between pre- and post-intervention score.

Adjusted intervention effects are presented in Table 3. The results of adjusted intervention effects, like unadjusted ones, were consistently beneficial and statistically significant ($p<0.05$) for the variables of knowledge about AEs of OPs, knowledge about self-protection from OP exposure, perceived susceptibility, and perceived barriers ($p<0.05$). The variables of perceived severity and perceived benefits statistically were significant after being adjusted for level of education and years working as a farmworker. On the other hand, variable of cues to action was not significant in both statistical analyses.

A comparison of Table 2 and Table 3 indicates that adjustment was significant in increasing the differences in modelled benefits of the intervention presented by both absolute magnitude and a percentage of the baseline mean scores.

Table 3: Absolute magnitudes of adjusted intervention effects on knowledge score and perceptions score, and intervention effects as percentages of baseline and follow-up scores.

Variables	Overall mean at baseline	Intervention effects (adjusted)		
		Follow-up		
		Absolute magnitude (95% CI)	*p-value*	As % of baseline mean
Score of knowledge about adverse effects of OPs	13.7	3.4 (2.3-4.5)	< 0.001	24.8
Score of knowledge about self-protection from OP exposure	14.8	1.9 (0.9-2.9)	< 0.001	12.8
Score of perceived susceptibility	20.9	2.8 (1.7-3.8)	< 0.001	13.4
Score of perceived severity	9.9	0.8 (0.3-1.3)	0.002	8.1
Score of perceived benefits	8.4	0.7 (0.1-1.4)	0.027	8.3
Score of perceived barriers	9.9	1.2 (0.7-1.6)	< 0.001	12.1
Score of cues to action	13.9	0.4 (-0.1-0.8)	0.102	2.9

Abbreviations: CI, confidence interval; OPs, organophosphate pesticides; OP, organophosphate.
Note: *p-value* refers to the difference between pre- and post-intervention score.

Adjusted mean scores of knowledge about AEs of OPs and knowledge about self-protection from OP exposure in Indonesian farmworkers and SA migrant farmworkers, at two measurement times, are shown in Figures 3 and 4 respectively.

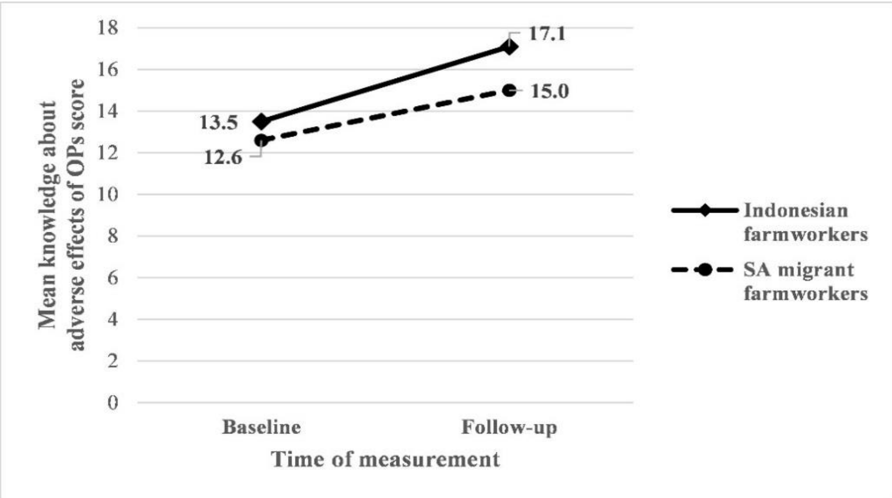

Figure 3: Adjusted mean score of knowledge about adverse effects of OPs in Indonesian farmworkers and SA migrant farmworkers at baseline and follow-up (*p<0.001*).
Notes: Scores were adjusted for level of education and years working as a farmworker. The follow up is at 3 months after the intervention (group communication in Indonesian farmworkers and one-on-one approach in SA migrant farmworkers).
Abbreviations: OPs, organophosphate pesticide; SA, South Australian.

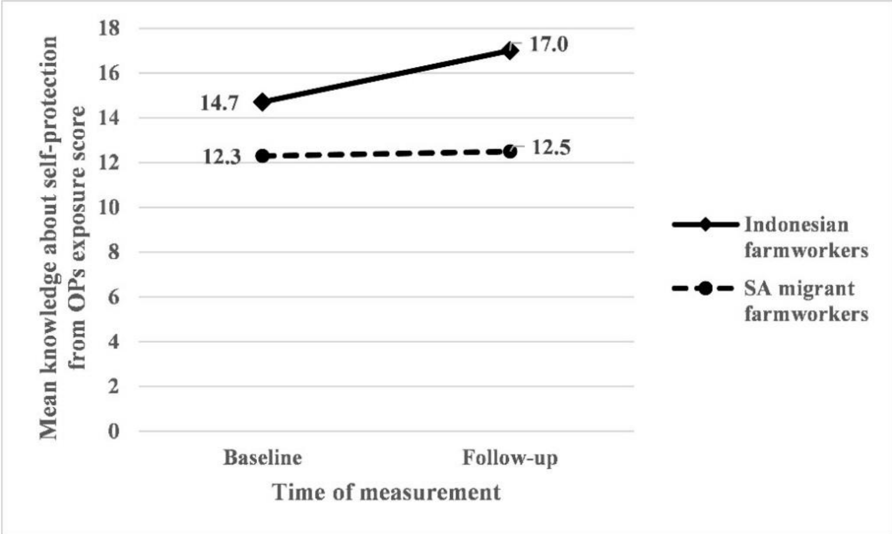

Figure 4: Adjusted mean score of knowledge about self-protection from OP exposure in Indonesian farmworkers and SA migrant farmworkers at baseline and follow-up (*p<0.001*). The follow up is at 3 months after the intervention (group communication in Indonesian farmworkers and one-on-one approach in SA migrant farmworkers).

Notes: Scores were adjusted for level of education and years working as a farmworker.

Abbreviations: OP, organophosphate; SA, South Australian.

Adjusted mean scores of perceptions about OP exposure in Indonesian farmworkers and SA migrant farmworkers, at two measurement times, are shown in Figures 5-9 (perceived susceptibility [Figure 5], perceived severity [Figure 6], perceived benefits [Figure 7], perceived barriers [Figure 8], and cues to action [Figure 9]).

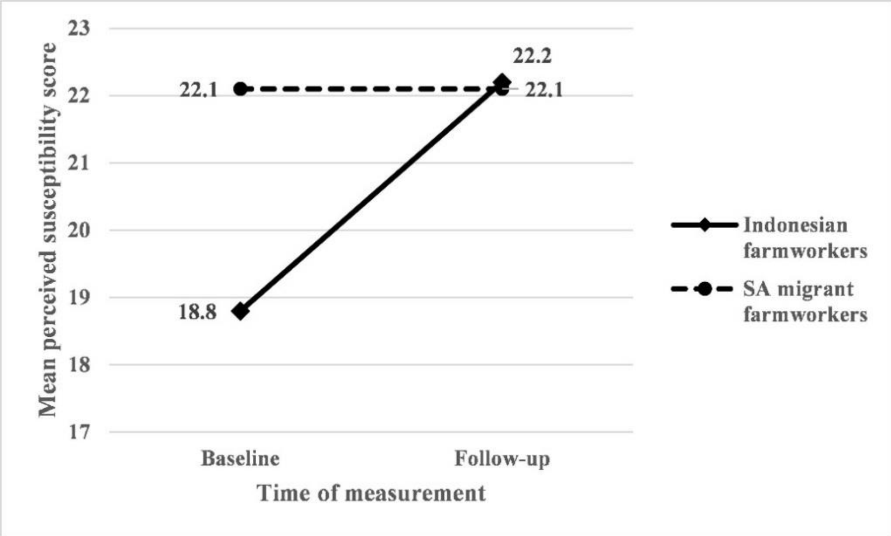

Figure 5: Adjusted mean score of perceived susceptibility in Indonesian farmworkers and SA migrant farmworkers at baseline and follow-up (*p<0.001*).

Notes: Scores were adjusted for level of education and years working as a farmworker. The follow up is at 3 months after the intervention (group communication in Indonesian farmworkers and one-on-one approach in SA migrant farmworkers).

Abbreviation: SA, South Australian.

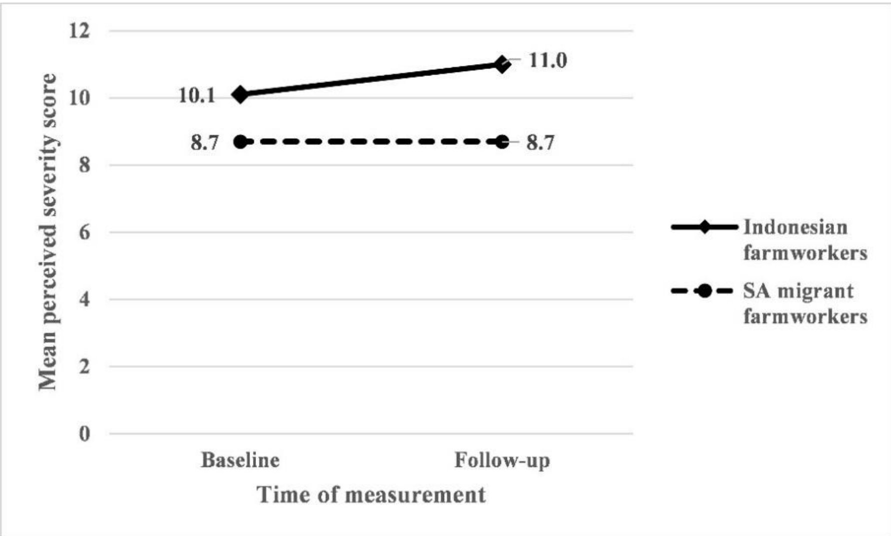

Figure 6: Adjusted mean score of perceived severity in Indonesian farmworkers and SA migrant farmworkers at baseline and follow-up (*p=0.002*).

Notes: Scores were adjusted for level of education and years working as a farmworker. The follow up is at 3 months after the intervention (group communication in Indonesian farmworkers and one-on-one approach in SA migrant farmworkers).

Abbreviation: SA, South Australian.

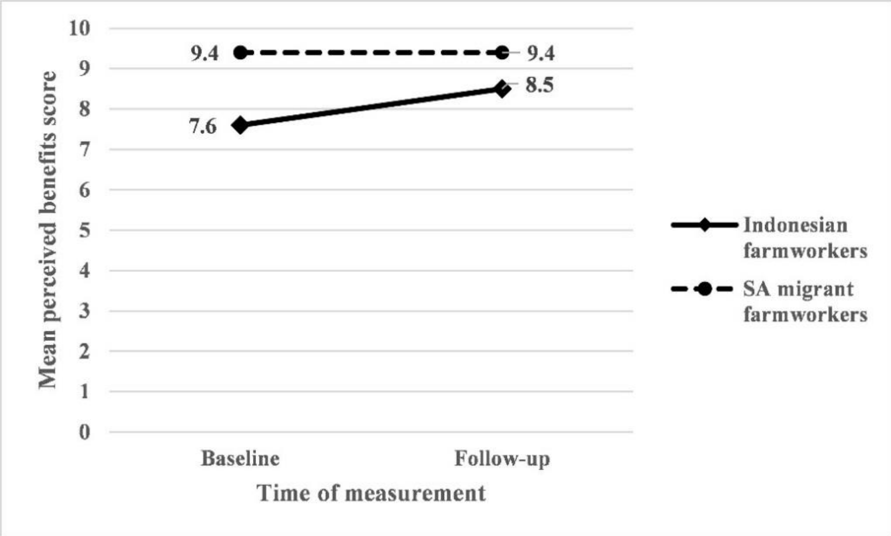

Figure 7: Adjusted mean score of perceived benefits in Indonesian farmworkers and SA migrant farmworkers at baseline and follow-up (*p=0.027*).

Notes: Scores were adjusted for level of education and years working as a farmworker. The follow up is at 3 months after the intervention (group communication in Indonesian farmworkers and one-on-one approach in SA migrant farmworkers).

Abbreviation: SA, South Australian.

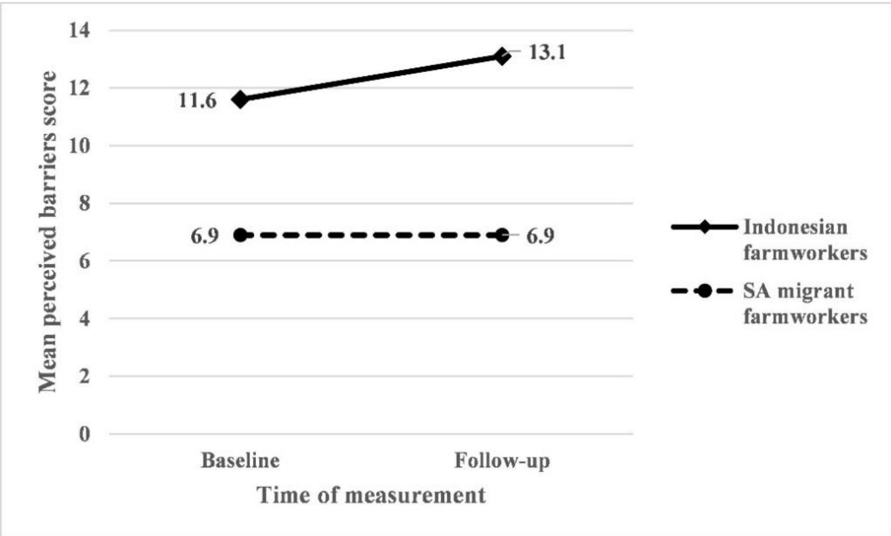

Figure 8: Adjusted mean score of perceived barriers in Indonesian farmworkers and SA migrant farmworkers at baseline and follow-up (*p<0.001*).

Notes: Scores were adjusted for level of education and years working as a farmworker. The follow up is at 3 months after the intervention (group communication in Indonesian farmworkers and one-on-one approach in SA migrant farmworkers).

Abbreviation: SA, South Australian.

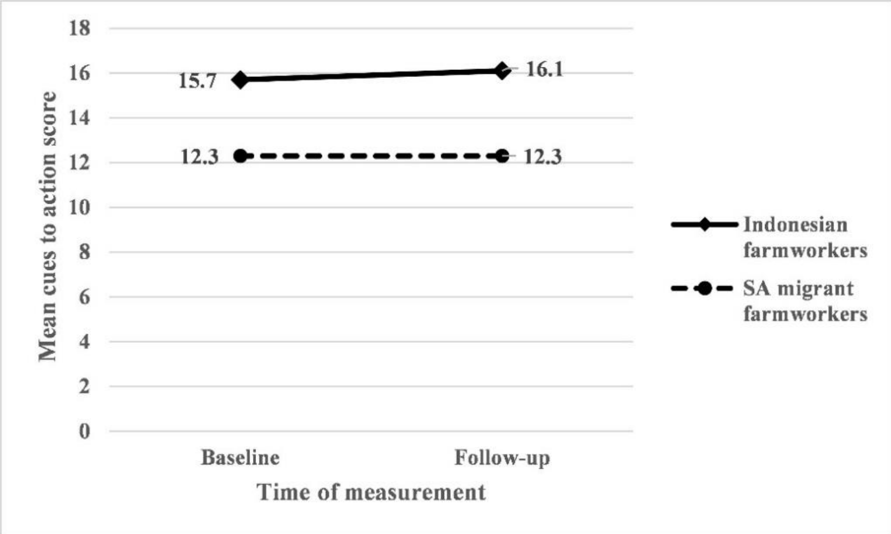

Figure 9: Adjusted mean score of cues to action in Indonesian farmworkers and SA migrant farmworkers at baseline and follow-up (*p>0.05*).
Notes: Scores were adjusted for level of education and years working as a farmworker. The follow up is at 3 months after the intervention (group communication in Indonesian farmworkers and one-on-one approach in SA migrant farmworkers).
Abbreviation: SA, South Australian.

These figures illustrate that the increases in both scores from baseline to follow-up were greater in Indonesian farmworkers. This demonstrates the beneficial effect of the intervention on both scores by using the method of presenting PowerPoint slides followed by discussion.

4. Discussion

This study found that locally tailored educational interventions improved the farmworkers' knowledge and perceptions of OP exposure after adjusting for level of education and years working as a farmworker (Table 3). The results of this study support those reported by Boonyakawee et al. (2013) in Thailand, which reported that farmworkers improved their knowledge after being provided training in insecticide-related knowledge. These results indicated that the objectives of the interventions were attained, except for cues to action. Knowledge about AEs of OPs and self-protection from OP exposure support the HBM. In the HBM theory, knowledge is one of the modifying factors that has a direct relationship with individual beliefs (perceived susceptibility, perceived severity, perceived benefits, and perceived barriers) and an indirect relationship with individual behaviours (Champion & Skinner, 2008). Knowledge of health risks and benefits of different health practices creates the precondition to practice health behaviour (Bandura, 2004).

This study found statistically significant improvements of farmworkers' knowledge (knowledge of OP toxicity, pathways of OP exposure at workplace and at home, signs and symptoms of acute and chronic effects due to OP exposure, self-protection from OP exposure at workplace and at home, PPE, and the first aid when exposed to OPs) and farmworkers' perceptions about OP exposure, including perceived susceptibility, perceived severity, perceived benefits, and perceived barriers after being provided the intervention using a group communication method among Indonesian farmworkers compared with SA migrant farmworkers that were provided the intervention using a one-on-one approach. The two of four major constructs of perception are perceived susceptibility and perceived severity (Champion & Skinner, 2008; Stretcher et al., 1997). Perceived susceptibility refers to person's subjective perceptions regarding the risk of health conditions. In the case of a medical illness, these dimensions include acceptance of a diagnosis, personalised forecast for the re-susceptibility, and susceptibility towards a disease in general (Brandt et al., 2001; Champion & Skinner, 2008; Clark & Houle, 2009). Feeling susceptible to a condition which leads to a serious disease can encourage farmworkers to change their behaviour (Champion & Skinner, 2008; Stretcher et al., 1997). It depends on one's belief of the effectiveness of the various measures available to reduce the threat of disease, or the perceived benefits in making health efforts. Meanwhile, perceived severity refers to feelings about the seriousness of the disease, including the evaluation of the clinical and medical consequences (e.g. death, disability, and pain) and social consequences that may occur (such as the effects on employment, family life, and social relationships) (Brandt et al., 2001; Champion & Skinner, 2008; Clark & Houle, 2009). Perceived barriers appear due to heightened view of potential negative aspects of health-related behaviour change. Factors, such as uncertainty, side effects, and questions about suitability, anxiety, and stress may act as a barrier to changing behaviour (Brandt et al., 2001; Champion & Skinner, 2008; Clark & Houle, 2009). In addition, according to the HBM theory, behaviour is also influenced by cues to action. Cues to action are events, things, or people that/who encourage or trigger people to change their behaviour by using appropriate reminder systems, promoting awareness, or providing information (Brandt et al., 2001; Champion & Skinner, 2008; Clark & Houle, 2009).

Indonesian farmworkers had significant improvement for almost all measured variables (knowledge and perceptions), except for cues to action. On the other hand, SA migrant farmworkers had significant improvement in mean score of knowledge about AEs of OPs whereas mean score of knowledge about self-protection from OP exposure had insignificant improvement and mean scores of all aspects of perceptions remained constant.

The intervention of health education provided to farmworkers in both groups was viewed as an innovation (World Health Organization, 2012). Different methods of educational interventions between groups might influence effectiveness of provided interventions (ILEP, 1998). Group intervention was used for Indonesian farmworkers whereas individual intervention was used for SA migrant farmworkers. Geographical area was the main reason for using different methods of educational interventions. In Indonesia, the research participants lived in the same village and were easily gathered together. In South Australia, the research participants did not live in the same area and only could be visited in their farm areas by making an appointment first.

During the intervention, the research participants in Indonesia were active and participants in all processes of the intervention, including listening, discussing, interacting, or explaining their experiences in using OPs. On the other hand, in South Australia, a one on one approach using a flipchart was the method used to convey information. All research participants in South Australia were Vietnamese, and were prone to be passive participants that means SA migrant farmworkers tended to be hesitant in asking a question during discussion session after providing educational intervention. This might be due to limited English language proficiency and therefore the participants might be hesitant in expressing their opinions in English language. The messages are much more effectively understood, when the target groups have an opportunity to express their opinions and interact (ILEP, 1998).

In Australia, the National Farmer's Federation (NFF) and the Rural Training Council of Australia (RTCA) conducted the national training and accreditation program for farm chemical users, known as ChemCert Australia to improve the knowledge, skills, attitude, and behaviour of farm chemical users (Radcliffe, 2002).

4.1. Methodological considerations

The intervention in this study was specifically targeted to reduce OP exposure. The sample was limited to one village of one regency in Indonesia (30 Indonesian farmworkers) and one region of one state in Australia (seven SA migrant farmworkers) due to difficulties in recruiting research participants in Australia. The intervention program only lasted for 1 hour, so possibly greater improvement post-intervention would have been observed had the educational intervention been delivered over a longer timeframe. In addition, this study only adjusted two factors, namely level of education and years of working as a farmworker, as covariates. We did not measure other external factors such as government awareness programs, information obtained from other sources such as the media, etc, which might influence the scores of knowledge and perceptions in the follow-up measurement. Moreover, self-reported data might occur in this study and might introduce potential bias like social desirability bias. Self-report often threaten the validity of research. Limited English language proficiency among SA migrant farmworkers might be a confounding variable that influenced the results of this study. Therefore, information bias might occur. The data collection times pre-intervention used in the two groups of farmworkers aligned with the times of spraying, otherwise the data collection times post-intervention used in a majority of Indonesian farmworkers did not align with the times of spraying due to the very dry season in Indonesia at 3 months after the intervention. Notwithstanding, the improvements resulted by the intervention in this study provide starting points to change behaviour of farmworkers, particularly to reduce OP exposure both at the workplace and at home.

5. Conclusions

Indonesian farmworkers had significant improvements in almost all aspects of knowledge and perceptions about OP exposure in the follow-up measurement after providing the interventions. In contrast, SA migrant farmworkers had insignificant improvements in all measured variables, except for knowledge about AEs of OPs. This might be due to the different methods of the interventions provided to both groups. The use of group communication was more effective to improve farmworkers' knowledge and perceptions than individual approach.

SA migrant farmworkers require a specific method of educational intervention to improve their knowledge and perceptions of OP exposure. Following ChemCert courses to obtain chemical accreditations conducted by ChemCert Training Group before working in agricultural sector is a suitable option to improve knowledge and the skills of SA migrant farmworkers in performing duties safely.

Further research needs to be conducted using long-term intervention methods, particularly for Indonesian farmworkers, to assess the effectiveness of interventions associated with changes of health behaviour outcomes.

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Disclosure

The authors report no conflicts of interest in this work.

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Appendices

Appendix A - Knowledge about adverse effects of OPs (12 questions total)

Check only one choice in each question. (Correct answers are checked. Correct answers received 2 points, “don’t know” answers received 1 point, and incorrect answers received 0 point. Minimum and maximum possible total scores were 0 and 24 respectively).

Q #	Statements	T	F	DK
A1	OP is not one of the insecticide types	☐	√	☐
A2	Fungicides are more toxic than insecticides	☐	√	☐
A3	Insecticides are not harmful for human health	☐	√	☐
A4	Farmworkers can suffer from pesticide poisoning when they are applying OPs on crops	√	☐	☐
A5	OPs can enter the body through inhalation	√	☐	☐
A6	Headache, nausea, cough, and sore throat after applying OPs on crops are not symptoms of pesticide poisonings	☐	√	☐
A7	Vomiting, sweating, chest pain, and diarrhoea are the symptoms of mild pesticide poisoning	☐	√	☐
A8	Pesticide poisonings can occur even when farmworkers wash their hands before eating and drinking	√	☐	☐
A9	OPs will not cause death unless it is swallowed	☐	√	☐
A10	Psychic disturbances or hallucinations are not symptoms of pesticide poisonings	☐	√	☐
A11	Risk of pesticide poisoning can be reduced by washing hands using clean water and soap before eating and drinking	√	☐	☐
A12	OP insecticides are the most toxic pesticides	√	☐	☐

Abbreviation: T, true; F, false; DK, don’t know; OPs, organophosphate pesticides; OP, organophosphate

Appendix B - Knowledge about self-protection from OP exposure (10 questions total)

Check only one choice in each question. (Correct answers are checked. Correct answers received 2 points, “don’t know” answers received 1 point, and incorrect answers received 0 point. Minimum and maximum possible total scores were 0 and 20 respectively).

Q #	Statements	T	F	DK
B1	Clothing contaminated by OPs is not a factor contributing to pesticide poisonings	☐	√	☐
B2	Smoking in the field raises the possibility of OPs entering the body	√	☐	☐
B3	Throwing away empty pesticide containers in a farm area is okay because it will not contaminate the environment	☐	√	☐
B4	Unused OPs must be stored in a ventilated room and separated from pantry or kitchen	√	☐	☐
B5	Re-entry into a farm area immediately after pesticide spraying without wearing PPE will increase amount of chemical materials absorbed by a human body	√	☐	☐
B6	Mixing OPs using bare hands is not harmful and will not cause adverse effects on human health	☐	√	☐
B7	Mostly farmworkers will not suffer from pesticide poisonings even though they do not wear PPE when working	☐	√	☐
B8	Wearing unwashed clothing after working in a farm area can be related to signs and symptoms of pesticide poisonings	√	☐	☐
B9	Pesticide poisonings may occur even if farmworkers shower immediately after working	√	☐	☐
B10	Wearing PPE is one of the ways to reduce and to prevent pesticide exposure during and after working in farm area	√	☐	☐

Abbreviation: T, true; F, false; DK, don’t know; OPs, organophosphate pesticides; PPE, personal protective equipment; OP, organophosphate

Appendix C – Perceptions about OP exposure (perceived susceptibility, perceived severity, perceived benefits, perceived barriers, and cues to action). Check only one choice for each question. (Positive-direction questions were scored from 1 point for 'strongly disagree' to 5 points for 'strongly agree'. Negative-direction questions were scored from 1 point for 'strongly agree' to 5 points for 'strongly disagree').

Q #	Statements	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree	Direction of question
Perceived Susceptibility (6 questions total) **Minimum possible scores = 6; Maximum possible scores = 30**							
C1	Exposure to OPs will not cause any adverse effects to me	☐	☐	☐	☐	☐	Negative
C2	Other farmworkers may suffer from pesticide poisoning	☐	☐	☐	☐	☐	Positive
C3	Human skin is not a route of OPs to enter the body	☐	☐	☐	☐	☐	Negative
C4	OPs are not dangerous for the human body	☐	☐	☐	☐	☐	Negative
C5	OPs are not harmful to the body as long as they are not swallowed	☐	☐	☐	☐	☐	Negative
C6	Following pesticide exposure, the pesticide is removed by the liver	☐	☐	☐	☐	☐	Positive
Perceived Severity (4 questions total) **Minimum possible scores = 4; Maximum possible scores = 20**							
C7	If the pesticide is on the skin, it will only cause a mild effect and it will recover soon	☐	☐	☐	☐	☐	Negative
C8	OPs only cause itchy skin	☐	☐	☐	☐	☐	Negative
C9	The effect of pesticide on the body is easily cured	☐	☐	☐	☐	☐	Negative
C10	Redness on the skin after working with OPs in the fields is not harmful because it is only as an effect of sunlight exposure	☐	☐	☐	☐	☐	Negative

(Appendix C: Continued)

Q #	Statements	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree	Direction of question
Perceived Benefits (2 questions total) **Minimum possible scores = 2; Maximum possible scores = 10**							
C11	Use of PPE will protect the body from adverse effects of pesticide exposure	☐	☐	☐	☐	☐	Positive
C12	Although a bit troublesome, wearing PPE is necessary to improve health	☐	☐	☐	☐	☐	Positive
Perceived Barriers (4 questions total) **Minimum possible scores = 4; Maximum possible scores = 20**							
C13	Use of PPE is troublesome	☐	☐	☐	☐	☐	Negative
C14	PPE is expensive	☐	☐	☐	☐	☐	Negative
C15	Use of PPE causes an uncomfortable feeling in the work	☐	☐	☐	☐	☐	Negative
C16	Following all pesticide safety procedures is not efficient because it will need extra time to finish my farm work	☐	☐	☐	☐	☐	Negative
Cues to Action (4 questions total) **Minimum possible scores = 4; Maximum possible scores = 20**							
C17	A health worker often reminds me to use PPE when I am working	☐	☐	☐	☐	☐	Positive
C18	My friends were ever sick due to not following pesticide safety procedures during work	☐	☐	☐	☐	☐	Positive
C19	My body often feels itchy after using OPs without wearing PPE	☐	☐	☐	☐	☐	Positive
C20	I often feel dizzy after spraying OPs on crops	☐	☐	☐	☐	☐	Positive

Abbreviation: OPs, organophosphate pesticides; PPE, personal protective equipment

Chapter 4. Field Practices in Handling OPs

Differences in Practices of Handling Organophosphate Pesticides (OPs) and OP-related Symptoms between Indonesian and South Australian Migrant Farmworkers: Pre and Post Educational Intervention

Suratman[1,2], **Kirstin Ross**[1], **Kateryna Babina**[1], **John William Edwards**[1]

1. Health and Environment Group, School of the Environment, Faculty of Science and Engineering, Flinders University, Adelaide, SA, Australia.
2. School of Public Health, Faculty of Health Sciences, Jenderal Soedirman University, Kampus Karangwangkal, Purwokerto 53122, Indonesia.

Keywords

Educational intervention; field practices; organophosphate pesticides-related symptoms; Indonesian farmworkers; South Australian migrant farmworkers.

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Abstract

Objective: The aim of this study was to describe the differences of field practices in handling OPs and the prevalence of OP-related symptoms among Indonesian and South Australian (SA) migrant farmworkers between pre and post educational intervention. Study design: This was a quasi-experimental study. Methods: Thirty Indonesian farmworkers at Dukuhlo Village, Brebes Regency, Indonesia, were provided the educational intervention using a method of group communication whereas seven migrant farmworkers in Virginia, South Australia were provided the educational intervention individually. Data were collected by interview using a structured questionnaire. Results: Some significantly behavioural improvements (p<0.05) in handling OPs as the result of the intervention occurred among Indonesian farmworkers as follows: 1) proportions of farmworkers who were touching crops after OP application dramatically decreased from 63% in pre intervention to 17% in post intervention; 2) proportions of farmworkers who were spraying OPs against wind direction sharply declined from 60% in pre intervention to 30% in post intervention; 3) proportions of farmworkers who were avoiding spray drift when applying OPs dramatically rose from 47% in pre intervention to 97% in post intervention; 4) proportions of farmworkers who were ensuring to not affect other people by over applied spray drift when applying OPs sharply increased from 53% in pre intervention to 93% in post intervention; and 5) proportions of farmworkers who were suffering from OP-related symptoms slightly decreased from 67% in pre intervention to 63% in post intervention. On the other hand, field practices of SA migrant farmworkers in post educational intervention remained constant as they did in pre intervention. Conclusions: Provision of appropriate equipment and long-term educational intervention linked to workplace was needed to

improve their knowledge, perceptions, and work practices to reducing adverse effects due to OP exposure.

1. Introduction

Organophosphate pesticide (OP)-related symptoms have been investigated in both developing and developed countries. In developing countries, a study by Kishi et al. (1995) in Indonesia found that 21% of 204 OP sprayers in Tegal and Brebes Regency had at least four symptoms related to OP exposure, including neurobehavioral, gastrointestinal, and respiratory symptoms. Similarly, Dasgupta et al. (2007) in the Mekong Delta in Vietnam reported that all 190 farmworkers assessed had some symptoms after mixing and spraying OPs. These symptoms consisted of skin irritation (66%), headache (61%), dizziness (49%), eye irritation (56%), and shortness of breath (44%). Rajashekhara et al. (2013) found that 25% of 76 patients working as agricultural workers and admitted to Jawaharlal Nehru Medical College were suffering from congested conjunctiva (87%), pin point pupil (83%), lacrimation (80%), vomiting (78%), non-reactive pupil (75%), respiratory distress (60%), and abdominal pain (37%). In developed countries, a study conducted by Strong et al. (2004) in eastern Washington State, (U.S.) among 211 farmworkers showed that the most common symptoms due to OP exposure reported by the participants were: headaches (50%), burning eyes (39%), pain in muscles, joints, or bones (35%), a rash or itchy skin (25%), and blurred vision (23%). Johnstone (2006) in Australia, assessing 50 farmworkers found that headache (2%), fatigue (3.9%), and watery eyes (3.9%) were the most frequently experienced symptoms reported by farmworkers due to OP exposure.

Field practices like mixing and spraying pesticides, use of personal protective equipment (PPE), washing hands or taking a shower after applying OPs, wearing contaminated clothes, eating, drinking and smoking during working with OP compounds were the most common factors contributing to OP exposure among farmworkers in developing and developed countries (Afriyanto, 2008; Arcury et al., 2002; Bradman et al., 2009; Johnstone, 2006; Lein et al., 2012; Mancini et al., 2009; Panuwet et al., 2008; Ribeiro et al., 2012; Shomar et al., 2014; Zhang et al., 2011).

This study was conducted to further understand the results of a study by Suratman et al. (2016, Chapter 3) among 30 Indonesian farmworkers and 7 SA migrant farmworkers that reported knowledge and perceptions of OP exposure among farmworkers in both countries. Comparisons of field practices in handling OPs and the prevalence of OP-related symptoms among farmworkers in both countries between pre and post educational intervention had not been previously investigated. Here we present results of a comparison of field practices in handling OPs and the prevalence of OP-related symptoms among Indonesian and SA migrant farmworkers, pre and post educational intervention. The educational intervention (described in detail in Suratman et al. (2016, Chapter 3)) was a short (one hour) delivery of information using group approach for Indonesian farmworkers and individual approach for SA migrant farmworkers relating to pesticide exposure, including definition of pesticides, groups of pesticides, pathways of OP exposure at workplace and at home, adverse health effects of OPs, signs and symptoms of acute and chronic effects due to OP exposure, self-protection from OP exposure at workplace, self-protection from OP exposure at home, personal protective equipment (PPE), and first aid when exposed to OP exposure.

2. Study Design and Methods

2.1. Study Population

This was a quasi-experimental study conducted in two research sites, Virginia, South Australia, Australia from May 2014 to June 2014 (pre educational intervention) and from September 2014 to October 2014 (post educational intervention); and at Dukuhlo Village, Brebes Regency, Central Java province, Indonesia from July 2014 to August 2014 (pre educational intervention) and from November 2014 to December 2014 (post educational intervention). Inclusion criteria of population were: 1) male; and 2) had to be employed in farm work within the past 3 months. Ethics approvals were obtained from Southern Adelaide Clinical Human Research Ethics Committee (SACHREC) with approval number: 319.13 and from Commission on Health Research Ethics, Faculty of Public Health, Diponegoro University, Semarang, Indonesia with approval number: 183/EC/FKM/2013.

Brebes Regency in Indonesia and Virginia in South Australia in this study were chosen as study sites conducted by Suratman et al. (2016, Chapter 3). Thirty Indonesian farmworkers and seven SA migrant farmworkers involved in this study were the same as the research participants in a study conducted by Suratman et al. (2016, Chapter 3). The ethnicity of the SA migrant farmworkers is Vietnamese.

2.2. Research Questionnaire Instrument

Data collection used an interviewer-administered questionnaire. The questionnaire was written in English and Indonesian. Questions were constructed based on the following literature: Workplace Health and Safety Queensland (2012); and other studies (Atreya, 2007; Berlin et al., 1980; Johnstone, 2006; LePrevost et al., 2011; Strong et al., 2008; Yassin et al., 2002; Zhang et al., 2011). The questionnaire consisted of: 1) activities associated with OP application as assessed by

5 closed-ended questions; 2) methods of OP application as assessed by 10 close-ended questions; 3) types of PPE usually worn when working with OPs as assessed by 6 closed-ended questions; 4) personal hygiene behaviour when working with OPs as assessed by 4 close-ended questions; 5) types of packaging and active ingredients of OPs products as assessed by 2 close-ended questions and 1 open-ended question; 6) workplace conditions as assessed by 9 close-ended questions; 7) OP-related symptoms as assessed by 16 symptoms questions.

Thirty Indonesian farmworkers were provided the intervention using a method of group communication whereas seven SA migrant farmworkers were provided the intervention individually. The intervention program in each group lasted for one hour. The provided educational intervention covered the following: 1) definition of pesticides; 2) groups of pesticides; 3) pathways of OP exposure at workplace and at home; 4) adverse health effects of OPs; 5) signs and symptoms of acute and chronic effects due to OP exposure; 6) self-protection from OP exposure at workplace; 7) self-protection from OP exposure at home; 8) personal protective equipment (PPE); and 9) first aid when exposed to OP exposure. Greater detail about study sites, study participants, and the contents of the educational intervention is presented in Suratman et al. (2016, Chapter 3).

2.3. Data Collection

The questionnaire was administered by an interviewer, face-to-face. This method was selected to obtain more accurate and complete answers, as the interviewer could clarify questions and responses at the same time. In South Australia, data collection was conducted from May to June 2014 for pre-intervention measurements and from September to October 2014 for post-intervention measurements. In Indonesia, data collection was conducted from July to August 2014 for pre-intervention measurements and from November to December 2014 for post-intervention measurements.

2.4. Data Analysis

Data were analysed using the statistical package SPSS. Categorical data were expressed as frequencies and proportions and were analysed using McNemar Test (Sheskin, 2004).

3. Results

3.1. Activities associated with OP application

Table 1 presents activities associated with OP application by Indonesian and SA migrant farmworkers between pre and post educational intervention. Generally, both groups did not differ in terms of the activities relating to OP application between two measurements.

3.2. Methods of OP application

Some methods of OP application among Indonesian and SA migrant farmworkers are presented in Table 2. Indonesian farmworkers usually used backpack sprayer to apply OPs to their crops (100%) in both measurements, poured OPs into the application tank using equipment such as bucket, dipper, cup, tablespoon, and trowel (90%), and used equipment to stir the mixture when mixing OPs such as dipper, tablespoon, and trowel (97%). On the other hand, a large majority of SA migrant farmworkers reported the use of hand spray gun (86%) to apply OPs, used their hands to pour the chemicals into tank (71%), and used their hands/arms (43%) and stick/paddle (43%) to stir in both measurements.

Meanwhile, nearly all (97%) of Indonesian farmworkers did not ride on equipment when applying OPs in pre intervention and all Indonesian farmworkers did not ride on equipment when applying OPs in post intervention. In contrast, 29% of SA migrant farmworkers rode a towing vehicle for applying OPs in both measurements. Riding on equipment means farmworkers was driving a tractor, a truck pulling a sprayer, a tank of pesticides, or another type of vehicle in the field. All the research participants in SA cultivated plants in a greenhouse. During a spraying period, they mixed chemical pesticides in a big container (a tank) in a chemical shed. They transported it from their chemical sheds to their greenhouses by driving a vehicle. In a greenhouse, they sprayed by hand. All research participants in both groups (100%) sprayed OPs on their crops in both pre and post intervention. More than 50% of Indonesian farmworkers were against wind direction when spraying OPs in pre intervention, whereas only 30% of them were upwind when spraying OPs in post intervention. On the other hand, all SA migrant farmworkers (100%) sprayed OPs following wind direction in both measurements. More than 50% of Indonesian farmworkers did not avoid spray drift when spraying OPs in pre intervention, whereas only 3% of them did not avoid spray drift when spraying OPs in post intervention. Approximately 47% of Indonesian farmworkers did not ensure that other people were not affected by applied spray drift, whereas in post intervention, only 7% of them did not ensure that other people were not affected by applied spray drift. In contrast, a large majority of SA migrant farmworkers (86%) avoided spray drift and also ensured that other people were not affected by spray drift in both pre and post intervention.

Table 1: Activities associated with OP application by Indonesian and SA migrant farmworkers.

Activity	Indonesian farmworkers (n=30)		SA migrant farmworkers (n=7)	
	Pre	**Post**	**Pre**	**Post**
I personally mixed OPs for farm purposes in the last three months:				
▪ Yes	30 (100%)	22 (73%)	7 (100%)	7 (100%)
▪ No	0 (0%)	8 (27%)	0 (0%)	0 (0%)
I personally loaded pesticides for farm purposes in the last three months:				
▪ Yes	30 (100%)	22 (73%)	4 (57%)	4 (57%)
▪ No	0 (0%)	8 (27%)	3 (43%)	3 (43%)
I personally sprayed crops in the last three months:				
▪ Yes	30 (100%)	21 (70%)	5 (71%)	5 (71%)
▪ No	0 (0%)	9 (30%)	2 (29%)	2 (29%)
I touched crops or plants after pesticides had been applied in the last three months: *)				
▪ Yes	19 (63%)	5 (17%)	3 (43%)	3 (43%)
▪ No	11 (37%)	25 (83%)	4 (57%)	4 (57%)
I rode equipment, such as a tractor or harvester for farm purposes in the last three months:				
▪ Yes	9 (30%)	0 (0%)	4 (57%)	4 (57%)
▪ No	21 (70%)	30 (100%)	3 (43%)	3 (43%)

*) Statistically significant among Indonesian farmworkers ($p<0.05$)

Table 2: Methods of OP application by Indonesian and SA migrant farmworkers.

Activity	Indonesian farmworkers (n=30)		SA migrant farmworkers (n=7)	
	Pre	Post	Pre	Post
Methods usually used for applying OPs to crops				
▪ Distribute granules	1 (3%)	0 (0%)	0 (0%)	0 (0%)
▪ Backpack sprayer	30 (100%)	30 (100%)	0 (0%)	0 (0%)
▪ Hand spray gun	0 (0%)	0 (0%)	6 (86%)	6 (86%)
Ways to pour the chemicals into the application tank when mixing OPs				
▪ Pour into tank by hand	3 (10%)	6 (20%)	5 (71%)	5 (71%)
▪ Other	27 (90%)	24 (80%)	2 (29%)	2 (29%)
Kind of equipment usually used to stir the mixture when mixing OPs				
▪ Hand/Arm	1 (3%)	0 (0%)	3 (43%)	3 (43%)
▪ Stick/Paddle	0 (0%)	1 (3%)	3 (43%)	3 (43%)
▪ Automatic Stir	0 (0%)	0 (0%)	1 (14%)	1 (14%)
▪ Other	29 (97%)	29 (97%)	0 (0%)	0 (0%)
Used a towing vehicle, such as tractor, trailer, or truck when applying OPs				
▪ Yes	1 (3%)	0 (0%)	2 (29%)	2 (29%)
▪ No	29 (97%)	30 (100%)	5 (71%)	5 (71%)
Spraying OPs on crops				
▪ Yes	30 (100%)	30 (100%)	7 (100%)	7 (100%)
▪ No	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Ways to spray OPs on crops *)				
▪ Wind direction	12 (40%)	21 (70%)	7 (100%)	7 (100%)
▪ Against wind direction	18 (60%)	9 (30%)	0 (0%)	0 (0%)
Avoiding spray drift when applying OPs *)				
▪ Yes	14 (47%)	29 (97%)	6 (86%)	6 (86%)
▪ No	16 (53%)	1 (3%)	1 (14%)	1 (14%)
Ensuring to not affect other people by over applied spray drift when applying OPs *)				
▪ Yes	16 (53%)	28 (93%)	6 (86%)	6 (86%)
▪ No	14 (47%)	2 (7%)	1 (14%)	1 (14%)

*) Statistically significant among Indonesian farmworkers (p<0.05)

3.3. Types of PPE Usually Worn When Working with OPs

Types of PPE usually worn by farmworkers during working with OPs in pre and post educational intervention are shown in Table 3.

Table 3: Types of PPE usually worn by Indonesian and SA migrant farmworkers when working with OPs.

Types of PPE	Indonesian farmworkers (n=30)		SA migrant farmworkers (n=7)	
	Pre	**Post**	**Pre**	**Post**
Clothes:				
▪ Long sleeved shirt	26 (87%)	30 (100%)	2 (28%)	2 (28%)
▪ Short sleeved shirt	4 (13%)	0 (0%)	4 (58%)	4 (58%)
▪ Coveralls	0 (0%)	0 (0%)	1 (14%)	1 (14%)
▪ Long pants/Leg covering	13 (43%)	15 (50%)	3 (43%)	3 (43%)
▪ Shorts	17 (57%)	15 (50%)	7 (100%)	7 (100%)
Headwear:				
▪ Wide brim hat	18 (60%)	1 (3%)	1 (14%)	1 (14%)
▪ Cap	12 (40%)	29 (97%)	6 (86%)	6 (86%)
Footwear:				
▪ Chemically resistant boots or shoes	0 (0%)	0 (0%)	2 (29%)	2 (29%)
▪ Waterproof boots	0 (0%)	0 (0%)	5 (71%)	5 (71%)
▪ Sneaker	0 (0%)	0 (0%)	0 (0%)	0 (0%)
▪ No shoes	30 (100%)	30 (100%)	0 (0%)	0 (0%)
Mask:				
▪ Gas mask, Cartridge mask	0 (0%)	0 (0%)	5 (71%)	5 (71%)
▪ A filtering facepiece	2 (7%)	1 (3%)	1 (14%)	1 (14%)
▪ Other mask/respirators	3 (10%	1 (3%)	1 (14%)	1 (14%)
▪ No mask	25 (83%)	28 (93%)	0 (0%)	0 (0%)
Gloves:				
▪ Leather gloves	0 (0%)	0 (0%)	2 (29%)	2 (29%)
▪ Waterproof elbow length gloves	0 (0%)	0 (0%)	0 (0%)	0 (0%)
▪ Waterproof gloves	0 (0%)	1 (3%)	1 (14%)	1 (14%)
▪ Other types of gloves	2 (7%)	1 (3%)	2 (29%)	2 (29%)
▪ No gloves	28 (93%)	28 (93%)	2 (29%)	2 (29%)
Eye protections:				
▪ Safety glasses	1 (3%)	0 (0%)	5 (71%)	5 (71%)
▪ A face shield	0 (0%)	0 (0%)	0 (0%)	0 (0%)
▪ Chemical goggles	0 (0%)	0 (0%)	0 (0%)	0 (0%)
▪ Other types of eye protections	0 (0%)	0 (0%)	0 (0%)	0 (0%)
▪ No eye protection	29 (97%)	30 (100%)	2 (29%)	2 (29%)

In pre intervention, most of Indonesian farmworkers reported usually wore long sleeved shirt (87%) and wide brim hat (60%), and did not wear footwear (100%), mask (83%), gloves (93%), and eye protection (97%) when working with OPs. In post intervention, most of Indonesian farmworkers reported usually wore long sleeved shirt (100%) and cap (97%), and did not wear footwear (100%), mask (93%), gloves (93%), and eye protection (100%) when working with OPs. Meanwhile, most of SA migrant farmworkers in pre and post intervention reported usually wore short sleeved shirt (58%), shorts (100%), cap (86%), waterproof boots (71%), gas mask or cartridge mask (71%), leather gloves (29%), and safety glasses (71%) during working with OPs.

3.4. Personal Hygiene Behaviour When Working with OPs

Table 4 presents personal hygiene behaviour by Indonesian and SA migrant farmworkers when working with OPs in pre and post educational intervention.

Table 4: Personal hygiene behaviour of Indonesian and SA migrant farmworkers when working with OPs.

Activity	Indonesian farmworkers (n=30)		SA migrant farmworkers (n=7)	
	Pre	Post	Pre	Post
How often do you wash your hands after work using clean water and soap before eating?				
▪ Always	24 (80%)	30 (100%)	7 (100%)	7 (100%)
▪ Usually	0 (0%)	0 (0%)	0 (0%)	0 (0%)
▪ Sometimes	6 (20%)	0 (0%)	0 (0%)	0 (0%)
▪ Never	0 (0%)	0 (0%)	0 (0%)	0 (0%)
How often do you wash your hands after work using clean water and soap before touching regular clothes?				
▪ Always	27 (90%)	29 (97%)	6 (86%)	6 (86%)
▪ Usually	0 (0%)	1 (3%)	0 (0%)	1 (14%)
▪ Sometimes	2 (7%)	0 (0%)	1 (14%)	0 (0%)
▪ Never	1 (3%)	0 (0%)	0 (0%)	0 (0%)
How often do you take a shower immediately after work?				
▪ Always	26 (87%)	30 (100%)	4 (57%)	4 (57%)
▪ Usually	0 (0%)	0 (0%)	1 (14%)	1 (14%)
▪ Sometimes	4 (13%)	0 (0%)	0 (0%)	0 (0%)
▪ Never	0 (0%)	0 (0%)	2 (29%)	2 (29%)
How often do you wear the same clothes more than one day without washing them?				
▪ Always	24 (80%)	12 (40%)	2 (29%)	1 (14%)
▪ Usually	0 (0%)	0 (0%)	0 (0%)	0 (0%)
▪ Sometimes	0 (0%)	7 (23%)	0 (0%)	1 (14%)
▪ Never	6 (20%)	11 (37%)	5 (71%)	5 (71%)

Most Indonesian farmworkers (80%) reported always washing their hands using clean water and soap before eating in pre intervention and it increased to be 100% in post intervention measurement. In contrast, all SA migrant farmworkers (100%) reported always washing their hands using clean water and soap before eating in both sets of data. Similarly, most of the research participants among Indonesian farmworkers (90%) reported always washing their hands after work using clean water and soap before touching regular clothes in pre intervention and it increased to be 97% in post intervention. On the other hand, most of SA migrant farmworkers (86%) reported always washing their hands after work using clean water and soap before touching regular clothes in both sets of data.

The proportion of Indonesian farmworkers always taking a shower immediately after work increased from 87% in pre intervention to 100% in post intervention. In contrast, the proportion of its activity remained constant among SA migrant farmworkers in both sets of data (57%). The proportion of Indonesian farmworkers never wearing the same clothes more than one day without washing them increased from 20% in pre intervention to 37% in post intervention. In contrast, the proportion remained constant among SA migrant farmworkers in both sets of data (71%).

3.5. Types of Packaging and Active Ingredients of OP Pesticide Products

Table 5 presents types and active ingredients of OP pesticide products used by Indonesian and SA migrant farmworkers in pre and post educational intervention. Cans (41% and 87%) and bags (32% and 97%) were the most common types of OP pesticide packaging used by Indonesian farmworkers in both sets of data respectively. In contrast, most of the SA migrant farmworkers (86% and 100%) used liquid containers in both sets of data respectively. All research participants (100%) in both groups used insecticides in both pre and post intervention. However, fungicides and herbicides were also used by SA migrant farmworkers. Generally, chlorpyrifos was commonly used by approximately 50% of the research participants in Indonesian and SA migrant farmworkers in pre intervention. Meanwhile, triazophos was the other type of OP compounds commonly used by Indonesian farmworkers in pre intervention. The use of chlorpyrifos decreased to be 9% of Indonesian farmworkers in post intervention. The active ingredients in the OPs were identified by asking a question about brand names and codes of pesticide products that were used on crops by the research participants and confirming the OP compounds by reading all active constituents that were listed on the label in pesticide products shown by them. The percentage of the types of OPs used in Table 5 did not add to 100%. This was no use of OPs. In addition, in the post intervention period, the use of chlorpyrifos decreased to 9% that was due to no OP spraying needed.

Table 5: Types and active ingredients of OPs products used by Indonesian and SA migrant farmworkers.

Type and Active Ingredient	Indonesian farmworkers (n=30)		SA migrant farmworkers (n=7)	
	Pre	**Post**	**Pre**	**Post**
Types of OPs packaging:				
▪ Bags	18 (32%)	29 (97%)	0 (0%)	0 (0%)
▪ Cans	23 (41%)	26 (87%)	0 (0%)	0 (0%)
▪ Liquid containers	0 (0%)	1 (3%)	6 (86%)	7 (100%)
▪ Bottles	15 (27%)	1 (3%)	1 (14%)	1 (14%)
▪ Other types	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Types of Pesticides:				
▪ Insecticides	30 (100%)	30 (100%)	7 (100%)	7 (100%)
▪ Fungicides	30 (100%)	30 (100%)	6 (86%)	6 (86%)
▪ Herbicides	0 (0%)	0 (0%)	3 (43%)	3 (43%)
▪ Rodenticides	0 (0%)	0 (0%)	2 (29%)	2 (29%)
Active ingredients of OPs:				
▪ Chlorpyrifos	15 (50%)	3 (9%)	3 (43%)	3 (43%)
▪ Triazophos	7 (23%)	0 (0%)	0 (0%)	0 (0%)

3.6. Workplace Conditions

Table 6 presents details of provided facilities to support personal hygiene in workplaces of Indonesian and SA migrant farmworkers in pre and post educational intervention. Generally, water and cups to drink were available in farm areas in both groups in both measurements. Notwithstanding, majority of the Indonesian farmworkers did not have access to water, soap, or towels; and did not have facilities to wash hands, toilet, and break room in their fields in both measurements. In contrast, these facilities were available in all SA migrant farmworkers' workplaces.

Table 6: Workplace conditions of Indonesian and SA migrant farmworkers.

Facility	**Indonesian farmworkers (n=30)**		**SA migrant farmworkers (n=7)**	
	Pre	**Post**	**Pre**	**Post**
There is water for you to drink in the fields:				
▪ Yes	29 (97%)	30 (100%)	4 (57%)	4 (57%)
▪ No	1 (3%)	0 (0%)	3 (43%)	3 (43%)
There are enough cups provided to drink using a clean cup for each worker:				
▪ Yes	26 (87%)	30 (100%)	6 (86%)	6 (86%)
▪ No	4 (13%)	0 (0%)	1 (14%)	1 (14%)
There is water to wash your hands:				
▪ Yes	4 (13%)	0 (0%)	7 (100%)	7 (100%)
▪ No	26 (87%)	30 (100%)	0 (0%)	0 (0%)
Soap is available for handwashing:				
▪ Yes	1 (3%)	0 (0%)	7 (100%)	7 (100%)
▪ No	29 (97%)	30 (100%)	0 (0%)	0 (0%)
Single use towels are available for handwashing:				
▪ Yes	3 (10%)	0 (0%)	2 (29%)	2 (29%)
▪ No	27 (90%)	30 (100%)	5 (71%)	5 (71%)
Washing water is separated from drinking water:				
▪ Yes	11 (37%)	11 (37%)	7 (100%)	7 (100%)
▪ No	19 (63%)	19 (63%)	0 (0%)	0 (0%)
There is any break room to take a rest for meals:				
▪ Yes	10 (33%)	10 (33%)	7 (100%)	7 (100%)
▪ No	20 (67%)	20 (67%)	0 (0%)	0 (0%)
There is a toilet facility:				
▪ Yes	1 (3%)	0 (0%)	7 (100%)	7 (100%)
▪ No	29 (97%)	30 (100%)	0 (0%)	0 (0%)

3.7. OP-related Symptoms

OP-related symptoms are all symptoms reported by farmworkers after working with OPs like weakness, headache, dizziness, nausea, vomiting, diarrhoea, salivation, watery eyes, sweating, difficulty working, psychic disturbances, chest pain, blue lips, heart palpitations, and muscle twitching.

Table 7 presents at least two or more OP-related symptoms suffered by Indonesian and SA migrant farmworkers. As many as 67% of Indonesian farmworkers reported OP-related symptoms in pre intervention and 63% in post intervention whereas 14% of SA migrant farmworkers reported such symptoms in pre intervention and no one reported such symptoms in post intervention.

Table 7: OP-related symptoms among Indonesian and SA migrant farmworkers.

OP-related symptoms	Indonesian farmworkers (n=30)		SA migrant farmworkers (n=7)	
	Pre	Post	Pre	Post
Yes	20 (67%)	19 (63%)	1 (14%)	0 (0%)
No	10 (33%)	11 (37%)	6 (86%)	7 (100%)

4. Discussion

This study provides useful information on all aspects of field practices in handling OPs and OP-related symptoms in two different groups of farmworkers, Indonesian and SA migrant farmworkers between pre and post educational intervention.

Generally, activities in handling OPs were relatively similar between pre and post educational intervention in both farmworker groups. This result indicated that the educational intervention provided to both groups did not significantly change their behaviour to reduce OP exposure. However, the intervention had significantly improved some work practices ($p<0.05$) among Indonesian farmworkers in not touching crops after OP application (Table 1), spraying method (Table 2), avoiding spray drift when applying OPs (Table 2), and ensuring to not affect other people by over applied spray drift when applying OPs (Table 2). In addition, proportions of farmworkers who suffering from OP-related symptoms slightly decreased from 67% in pre intervention to 63% in post intervention (Table 7). In contrast, generally, field practices of SA migrant farmworkers in post educational intervention remained constant as they did in pre intervention.

A study by Suratman et al. (2016, Chapter 3) among the same research participants who were involved in this study reported that the simple educational intervention had significantly improved scores of knowledge, perceived susceptibility, perceived severity, perceived benefits, and perceived barriers, except for cues to action among Indonesian farmworkers after being adjusted for level of education and years working as a farmworker. In contrast, SA migrant farmworkers did not have statistically significant improvements in almost all measured variables, except for knowledge about adverse effects of OPs. According to Champion and Skinner (2008) in the Health Belief Model (HBM) Theory, knowledge directly relates to perceptions (perceived susceptibility, perceived severity, perceived benefits, and perceived barriers) and indirectly relates to health-related behaviours. Perceived susceptibility and perceived severity, the two of four major constructs of perception, play important roles in changing health behaviours (Champion & Skinner, 2008; Stretcher et al., 1997).

Regarding the conditions in which the research participants in both groups worked, farming methods used by Indonesian and SA migrant farmworkers were completely different. Farmworkers in Indonesia cultivated their crops using a method of outdoor growing (open farm) whereas SA migrant farmworkers planted their crops in greenhouse. In addition, farmworkers in Indonesia used conventional farming practices in cultivating their plants by using low-technology techniques that were more feasible to practice and cheaper. Indonesian farmworkers had built a strong knowledge base from practical experiences, gained over generations. This knowledge had to be valued for potential gain in farming. On the other hand, farmworkers in Australia used high-technology methods in growing their crops including high-technology equipment that needed to spend much cost. These differences in worksite conditions and in farming methods might play an important role in their behavioural intentions before deciding to change behaviour. If farmworkers believed that wearing PPE during working with OP compounds would make their life more protective from OP exposure and be beneficial to their health and believed that important people in their life wanted them to protect their selves, and they were capable of using less OP compounds due to their past behaviour and evaluation of internal and external control factors, then this would predict high intentions to reduce OP exposure by wearing PPE.

In tropical area like Indonesia, most of farmworkers were reluctant to wear adequate PPE during working in the field due to hot weather and expensive (Kishi et al., 1995). Notwithstanding, Indonesian government, particularly Ministry of Agriculture and Ministry of Health, has regulated the use of pesticides and wearing PPE during working with chemical compounds including OPs. Similarly, Australian government, particularly The National Occupational Health and Safety Commission, the Australian Pesticides and Veterinary Medicines Authority (APVMA) (previously known as the National Registration Authority), and the states have strictly regulated farm chemical users to minimise the risks of adverse effects due to farmworker exposure to hazardous substances, including OPs (Radcliffe, 2002). In addition, the national training and accreditation program for farm chemical users, known as ChemCert Australia, was conducted by the National Farmer's Federation (NFF) and the Rural Training Council of Australia (RTCA). One of the aims of these programs was to improve the knowledge, skills, attitude, and behaviour of farm chemical users (Radcliffe, 2002).

5. Conclusions

Generally, provided educational intervention did not significantly change field practices in handling OPs in both Indonesian and SA migrant farmworkers. However, some significant behavioural improvements ($p<0.05$) in handling OPs as the result of the intervention occurred among Indonesian farmworkers as follows: 1) proportions of farmworkers who touching crops after OP application dramatically decreased from 63% in pre intervention to 17% in post intervention; 2) proportions of farmworkers who spraying OPs against wind direction sharply declined from 60% in pre intervention to 30% in post intervention; 3) proportions of farmworkers who avoiding spray drift when applying OPs dramatically rose from 47% in pre intervention to 97% in post intervention; 4) proportions of farmworkers who ensuring to not affect other people by over applied spray drift when applying OPs sharply increased from 53% in pre intervention to 93% in post intervention; and 5) proportions of farmworkers who suffering from OP-related symptoms slightly decreased from 67% in pre intervention to 63% in post intervention. On the other hand, generally, field practices of SA migrant farmworkers in post educational intervention remained constant as they did in pre intervention.

Provision of appropriate equipment and long-term educational intervention linked to workplace conditions is needed to improve their knowledge, perceptions, and work practices to reducing adverse effects due to OP exposure.

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Declarations of Interest

The authors declare that they have no conflicts of interest to report.

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Chapter 5. Cholinesterase Activity Levels

Levels of Erythrocyte Acetylcholinesterase (EAChE) and Plasma Cholinesterase (PChE) among Indonesian and South Australian Migrant Farmworkers

Suratman[1,2], **Kirstin Ross**[1], **Kateryna Babina**[1], **John William Edwards**[1]

1. Health and Environment Group, School of the Environment, Faculty of Science and Engineering, Flinders University, Adelaide, SA, Australia.
2. School of Public Health, Faculty of Health Sciences, Jenderal Soedirman University, Kampus Karangwangkal, Purwokerto 53122, Indonesia.

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Erythrocyte acetylcholinesterase; Indonesian farmworkers; organophosphate; plasma cholinesterase; South Australian migrant farmworkers.

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Abstract

This study measured activity levels of erythrocyte acetylcholinesterase (EAChE) and plasma cholinesterase (PChE) in Indonesian and South Australian (SA) migrant farmworkers to assess exposure to organophosphate pesticides (OPs), pre and post educational intervention.

This was a quasi-experimental study conducted on 30 farmworkers at Dukuhlo Village, Brebes Regency, Indonesia and seven farmworkers working in suburb of Virginia, South Australia. These levels were measured from 10μL fingerprick blood samples using the Test-mate ChE field kit at baseline (pre-educational intervention) and at 3 months after the educational intervention.

Mean EAChE activity levels in post intervention (29.45 ± 3.68 U/g Hb) were higher than in pre intervention (26.33 ± 3.69 U/g Hb) among Indonesian farmworkers (p<0.05). There was no difference in EAChE activity levels among SA migrant farmworkers in both measurements (27.41 ± 3.77 U/g Hb and 27.34 ± 3.46 U/g Hb respectively). Mean PChE activity levels in Indonesian farmworkers (1.61 ± 0.39 U/mL and 1.62 ± 0.50 U/mL respectively) and SA migrant farmworkers (1.65 ± 0.39 U/mL and 1.80 ± 0.52 U/mL respectively) did not significantly differ between both measurements. However the difference of EAChE and PChE activity levels between pre and post educational intervention could be related to the time elapsed since last exposure and not to the intervention performed.

1. Introduction

In developing countries, the number of deaths due to pesticide poisoning has been estimated to be higher that of infectious diseases (Eddleston et al., 2002). Organophosphate pesticides (OPs) are the biggest cause of pesticide poisoning, with the most common OP compounds used by farmworkers being chlorpyrifos, diazinon, and malathion (Heide, 2007; Weiss et al., 2004; WHO, 2009). In this study, chlorpyrifos was the common active constituent of OPs used by Indonesian farmworkers and SA migrant farmworkers (50% and 43% respectively) (Suratman et al., 2015b, Chapter 4). Even though chlorpyrifos is assumed to be moderately toxic to humans, previous studies have indicated that long-term exposure to OP compounds caused adverse health effects like lung cancer (Alavanja et al., 2004), persistent developmental disorders (Office of Environmental Health Hazard, 2007), and autoimmune disorders (Repetto & Baliga, 1996).

OPs' toxicity is due to the inhibition of the neural target enzyme, acetylcholinesterase (AChE). Levels of erythrocyte (EAChE) and plasma cholinesterase (PChE) activity in blood samples are used as biomarkers of OP-related acetylcholinesterase inhibition and are used to monitor farmworkers at risk of OP exposure (Mason, 2000; Office of Pesticide Programs, 2000).

Several studies investigating levels of EAChE and PChE among farmworkers had been conducted in developing (Afriyanto, 2008; Catano et al., 2008; Cecchi et al., 2012; Dasgupta et al., 2007; Jintana et al., 2009; Kashyap, 1986) and developed countries (Benmoyal-Segal et al., 2005; Costa et al., 2012; Gonzalez et al., 2012; Hofmann et al., 2009; Lopez-Granero et al., 2014; Nomura et al., 1986; Sanchez-Santed et al., 2004; Tromm et al., 1992).

The aim of this study was to compare mean activity levels of EAChE and PChE due to OP exposure between pre and post educational intervention among

Indonesian farmworkers and South Australian (SA) migrant farmworkers. The educational intervention (described in detail in Suratman et al. (2016, Chapter 3)) was a short (one hour) delivery of information relating to pesticide exposure, including definition of pesticides, groups of pesticides, pathways of OP exposure in the workplace and at home, adverse health effects of OPs, signs and symptoms of acute and chronic effects due to OP exposure, self-protection from OP exposure in the workplace, self-protection from OP exposure at home, personal protective equipment (PPE), and first aid when exposed to OP exposure.

2. Study Design and Methods

2.1. Study Population

This was a quasi-experimental study conducted in two research sites, the suburb of Virginia, South Australia, Australia from May 2014 to June 2014 (pre educational intervention) and from September 2014 to October 2014 (post educational intervention); and at Dukuhlo Village, Brebes Regency, Central Java province, Indonesia from July 2014 to August 2014 (pre educational intervention) and from November 2014 to December 2014 (post educational intervention). Inclusion criteria of population were: 1) male; and 2) had to be employed in farm work within the past 3 months. Ethics approvals were obtained from Southern Adelaide Clinical Human Research Ethics Committee (SACHREC) with approval number: 319.13 and from the Commission on Health Research Ethics, Faculty of Public Health, Diponegoro University, Semarang, Indonesia, with approval number: 183/EC/FKM/2013.

Brebes Regency in Indonesia and the suburb of Virginia in South Australia were chosen as study sites (Suratman et al., 2016, Chapter 3; Suratman et al., 2015, Chapter 4). Thirty Indonesian farmworkers and seven SA migrant farmworkers involved in this study were the same as the research participants in a study conducted by Suratman et al. (2016, Chapter 3) and by Suratman et al. (2015, Chapter 4). The ethnicity of the SA migrant farmworkers was Vietnamese. In this study, the common active constituent of OPs used by both groups was chlorpyrifos (Suratman et al., 2015, Chapter 4).

2.2. Materials and Methods

EAChE and PChE levels were measured according to the manufacturers instruction of the Test-mate ChE Cholinesterase Test System® (EQM Research, 2011). Briefly, farmworkers washed their hands well using soap and water and further cleaned with an alcohol swab. Blood was sampled using a new spring operated lancet stick. The first drop of blood was wiped away with sterile gauze, and the second drop was collected into a single capillary tube. Excess blood was removed from the external surface of the capillary tube by rolling it across filter paper. The capillary tube was inserted in to the Test-Mate vial assay tube and immediately read using the Test-Mate machine for immediate analysis of EAChE and PChE.

Principles of analysis of EAChE and PChE are as follows:

a. To minimize false negative due to cholinesterase reactivation, EAChE and PChE levels are immediately specified by a Test-Mate ChE Cholinesterase Test System (Model 400) after blood sample collection based upon the Ellman method (Ellman et al., 1961; EQM Research, 2011).
b. The system requires 10µL fingerprick blood sample for each test. Acetylthiocholine (AcTC) or Butyrylthiocholine (BuTC) is hydrolysed by EAChE and PChE, producing carboxylix acid and thiocholine which react with dithionitrobenzoic acid (DTNB) as the Ellman reagent to form a yellow colour which is spectrophotometrically tested at 450 nm (EQM Research, 2011).

Principles of the Test-mate ChE and equation for measuring cholinesterase activity calculated by the photometric analyser are as follows (EQM Research, 2011):

$$\text{thiocholine ester (AcTC)} \xrightarrow{\text{Cholinesterase}} \text{thiocholine}$$

$$\text{thiocholine + DTNB} \longrightarrow \text{TNB-thiocholine + TNB (yellow)}$$

$$\text{U/mL blood} = \frac{\text{(A/min) (mL assay volume)}}{(\varepsilon,\ \text{mM}^{-1})\ \text{(cm light path) (mL blood)}}$$

2.3. Data Analysis

Statistical analyses were performed using the statistical package SPSS version 17 (SPSS Inc., Chicago, IL, USA). Graphs were created using GraphPad Prism v6.05 (GraphPad Software Inc., 2014). Continuous data were tested for normal distribution using the Shapiro-Wilk test (Elliot & Woodward, 2007; Razali & Wah, 2011). If a normal distribution was found, data were expressed as means and standard deviations and were analysed using parametric method (Paired t test, Pearson Product Moment test, and one sample t test), otherwise data were expressed as medians and ranges and analysed using non parametric methods (Wilcoxon test and Spearman's Rank Correlation test). Level of statistical significance was set at $\alpha = 0.05$.

Levels of EAChE and PChE are also presented in clinical categories (ranging from normal to severe inhibition) of EAChE and PChE activity levels. Table 1 presents clinical categories of inhibition of EAChE based on percentage of normal EAChE activity (taken as 31.4 U/g Hb) (EQM Research, 2011; Rajapakse et al., 2011) and grading of inhibition of PChE based on percentage of normal PChE activity taken as 2.55 U/mL (EQM Research, 2011; Rajapakse et al., 2011).

Table 1: Categories of EAChE and PChE activity levels (EQM Research, 2011; Rajapakse et al., 2011)

Clinical Categories of EAChE Activity	Percentage of Normal EAChE activity (Normal Taken as 31.4 U/g Hb)	EAChE Level (U/g Hb)
Normal	≥ 75%	≥ 23.6
Mild inhibition	30% - 74%	9.4 - 23.5
Moderate inhibition	10% - 29%	3.1 - 9.3
Severe inhibition	< 10%	< 3.1
Clinical Categories of PChE Activity	**Percentage of Normal PChE activity (Normal Taken as 2.55 U/mL)**	**PChE Level (U/mL)**
Normal	≥ 75%	≥ 1.91
Mild inhibition	30% - 74%	0.77 - 1.90
Moderate inhibition	10% - 29%	0.26 - 0.76
Severe inhibition	< 10%	< 0.26

3. Results

3.1. OP application

Indonesian and SA migrant farmworkers were asked to estimate the last time they applied OPs (defined as mixing, loading, and spraying). Figure 1 and Figure 2 present these estimates, pre and post educational intervention. Most Indonesian farmworkers applied OPs to their crops in the preceding 1 – 6 days (83%) in pre educational intervention measurement, compared with most of them applying OPs in the last 2-4 months (40%) in post educational intervention measurement. This difference between time of exposure pre and post intervention has implications for interpreting our data (discussed in detail below). Most of SA migrant farmworkers had used OPs in the last 2-4 months (43%) in both pre and post intervention measurements. The active constituent of OPs generally used by both groups was chlorpyrifos, about 50% and 43% (Indonesian and SA migrant farmworkers respectively).

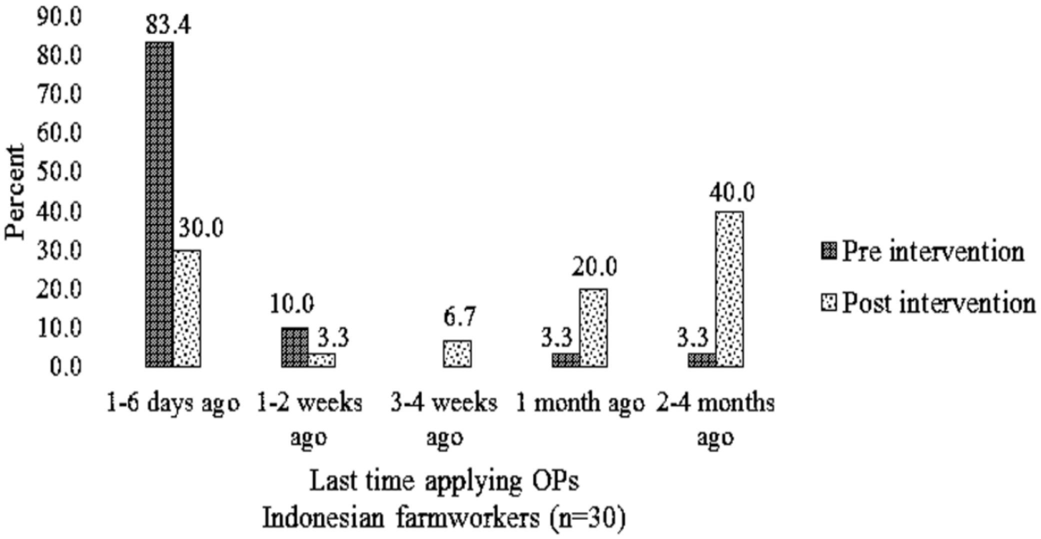

Figure 1: The last time applying OPs by Indonesian farmworkers in pre and post educational intervention measurements.

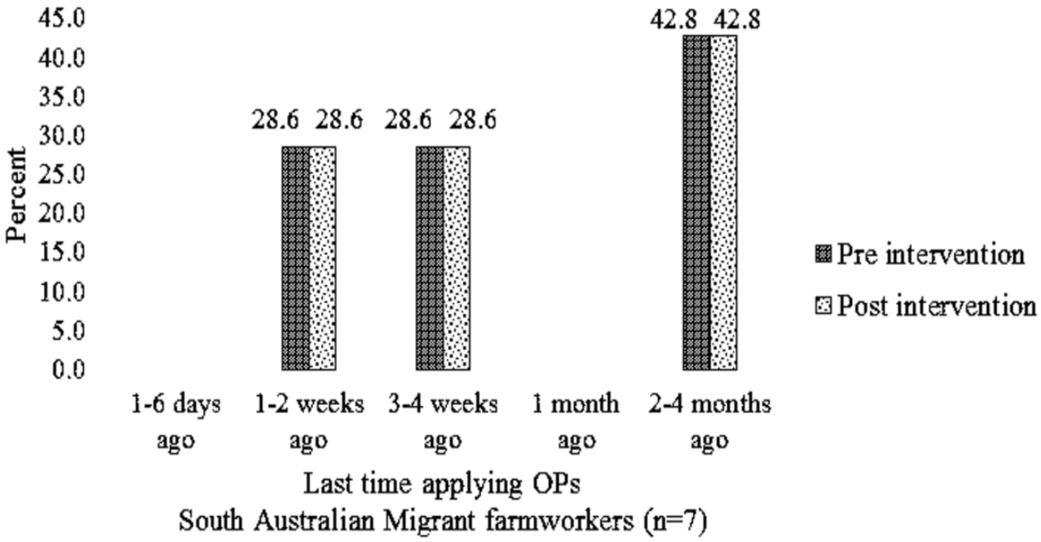

Figure 2: The last time applying OPs by SA migrant farmworkers in pre and post educational intervention measurements.

3.2. EAChE Activity Levels

Figure 3 presents activity levels of EAChE in individually matched fingerprick blood samples among Indonesian and SA migrant farmworkers pre and post educational intervention. Generally, EAChE activity levels of both groups slightly declined in the post intervention. However, EAChE activity levels in some Indonesian farmworkers decreased dramatically. There were statistically significant differences in EAChE activity levels between pre (29.45 ± 3.68 U/g Hb) and post (26.33 ± 3.69 U/g Hb) educational intervention measurements among Indonesian farmworkers (p<0.05). This contrast with EAChE activity levels among SA migrant farmworkers, which were not significantly different (p>0.05) between two measurement periods (27.41 ± 3.77 U/g Hb and 27.34 ± 3.46 U/g Hb respectively). In addition, the mean EAChE activity levels in both groups were statistically significantly lower than the reference population value (31.4 U/g Hb) using this kit, p<0.05.

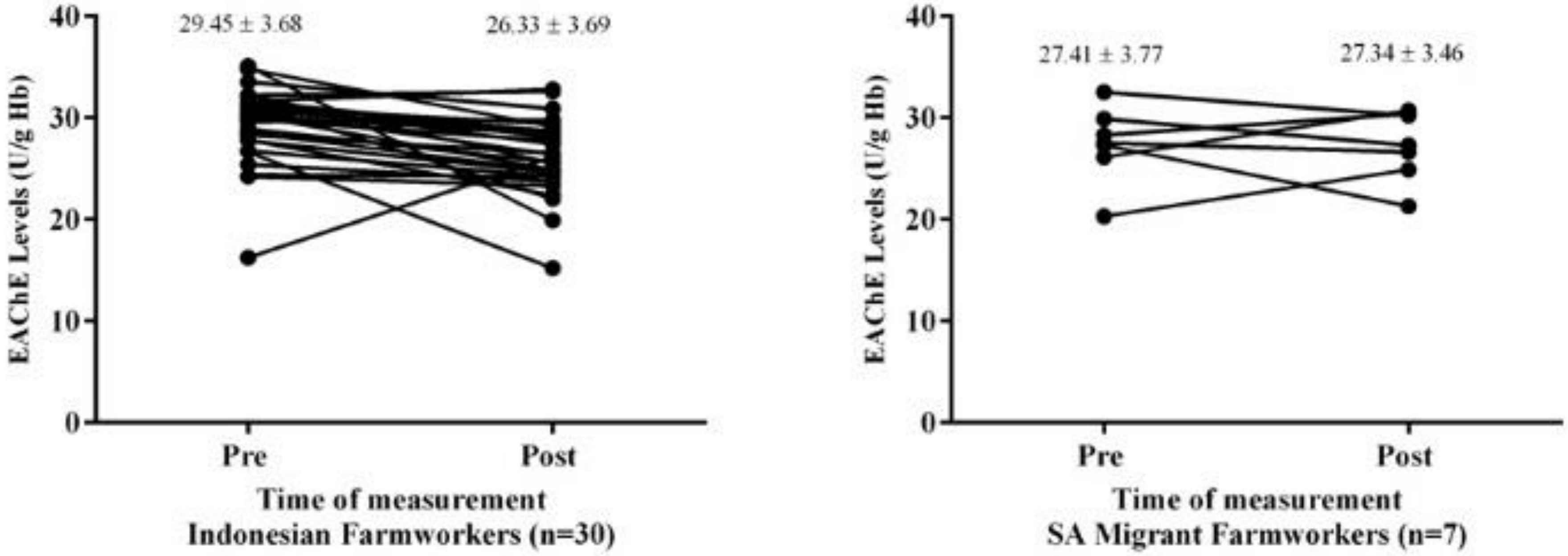

Figure 3: Activity levels of EAChE in individually matched fingerprick blood samples in pre and post educational intervention among Indonesian (p<0.05) and SA migrant farmworkers (p>0.05).
Note: The data labels used in Figure 3 were mean ± SD.
Abbreviation: SD, standard deviation.

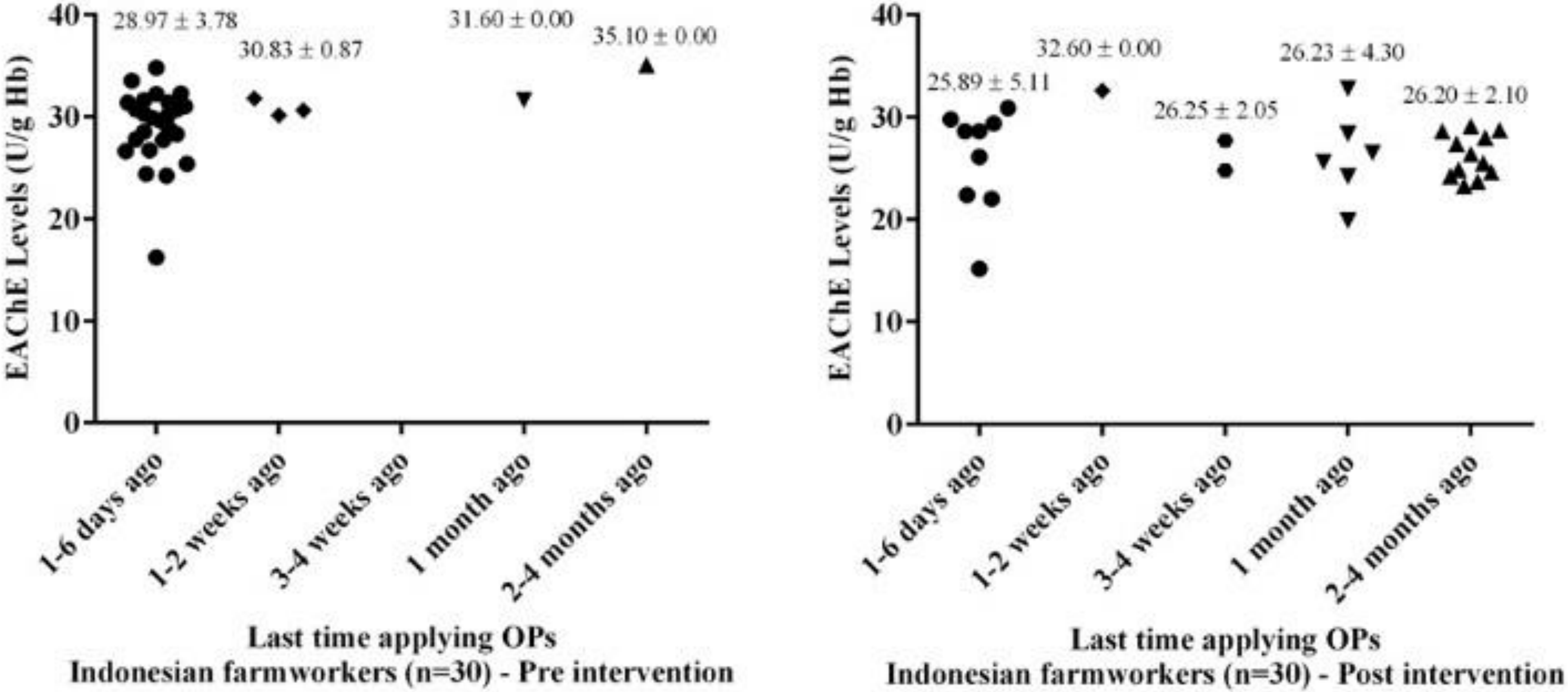

Figure 4: Last time applying OPs against EAChE activity levels in pre and post educational intervention among Indonesian farmworkers.
Note: The data labels used in Figure 4 were mean ± SD.
Abbreviation: SD, standard deviation.

Figure 4 shows the relationship between reported last time applying OPs and EAChE activity levels in pre and post educational intervention among Indonesian farmworkers. In the pre intervention, mean EAChE activity levels tended to be higher among farmworkers who applied OPs more than a month ago. There was no clear pattern of EAChE activity levels among farmworkers who applied OPs in various latest application time in the post intervention. The results of Spearman's Rank test indicated that there was no statistically significant correlation between the last time applying OPs and EAChE activity levels in both pre and post intervention (p>0.05).

Figure 5 presents the last time applying OPs and EAChE activity levels pre and post educational intervention among SA migrant farmworkers. In the pre intervention, mean EAChE activity levels tended to increase among farmworkers who applied OPs more than a month ago. As with the Indonesian farmworkers, in the post intervention, there was no pattern of EAChE activity levels among farmworkers who applied OPs in various latest application time. The results of Spearman's Rank test indicated that there was no statistically significant correlation between the last time applying OPs and EAChE activity levels in both pre and post intervention (p>0.05).

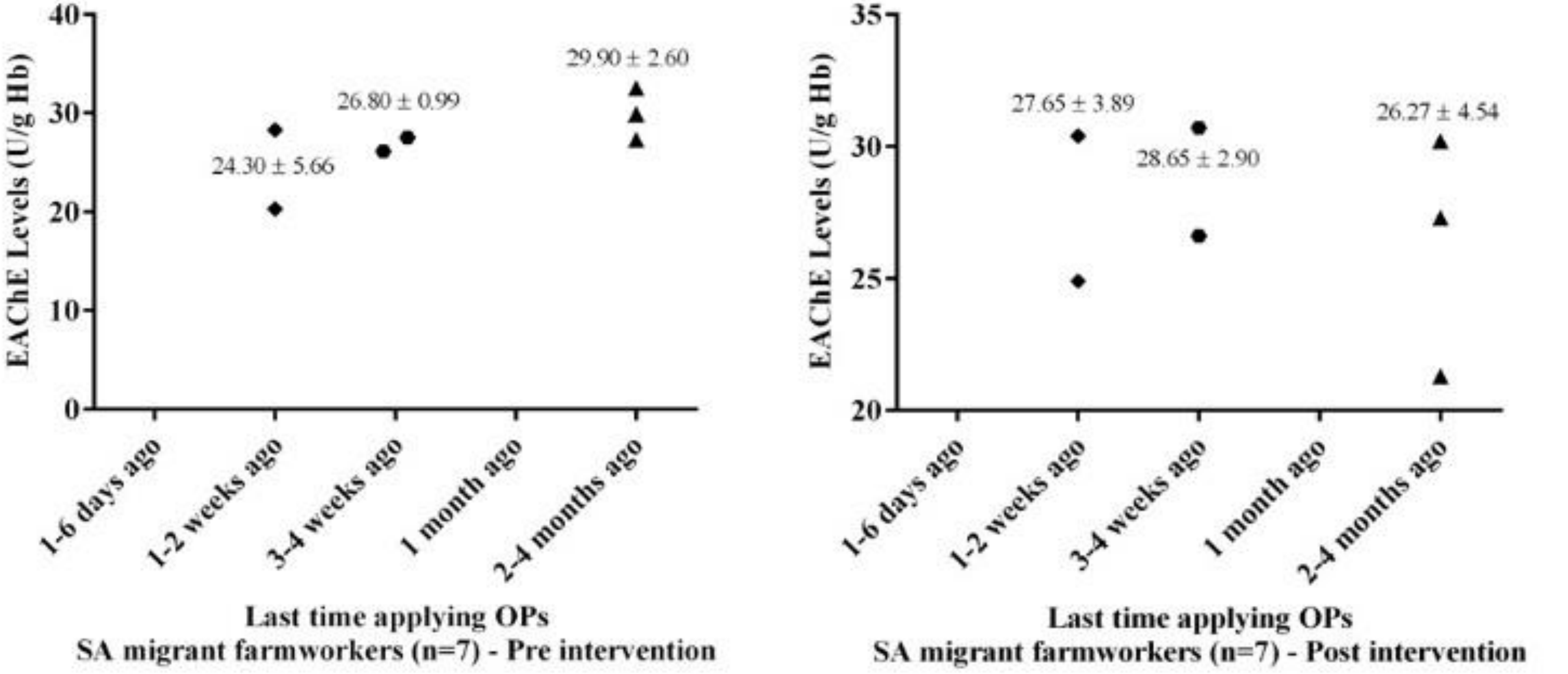

Figure 5: Last time applying OPs against EAChE activity levels in pre and post educational intervention among SA migrant farmworkers.
Note: The data labels used in Figure 5 were mean ± SD.
Abbreviation: SD, standard deviation.

3.3. PChE Activity Levels

Figure 6 presents activity levels of PChE in individually matched blood samples among Indonesian and SA migrant farmworkers. Generally, mean PChE activity levels in Indonesian farmworkers (1.61 ± 0.39 U/mL and 1.62 ± 0.50 U/mL respectively) and in SA migrant farmworkers (1.65 ± 0.39 U/mL and 1.80 ± 0.52 U/mL respectively) were slightly higher in the post intervention than those taken pre educational intervention. However, PChE activity levels in some Indonesian farmworkers and in one SA migrant farmworker decreased sharply. Overall, the results of paired t tests showed that there were no statistically significant differences in PChE activity levels between pre and post intervention in both groups ($p>0.05$). In addition, the mean PChE activity levels in both study groups were statistically significantly lower than the reference population value (2.55 U/mL) ($p<0.05$).

Figure 7 presents the last time applying OPs against PChE activity levels pre and post educational intervention among Indonesian farmworkers. In the pre intervention, mean PChE activity levels varied among farmworkers with various latest OP application time. For example, a farmworker who applied OPs more than a month ago had the highest mean levels of PChE activity levels. Similarly, there was no specific pattern of PChE activity levels among farmworkers who applied OPs in various latest application time in the post intervention. The results of Spearman's Rank test indicated that there was no statistically significant correlation between the last time applying OPs and PChE activity levels in both pre and post intervention ($p>0.05$).

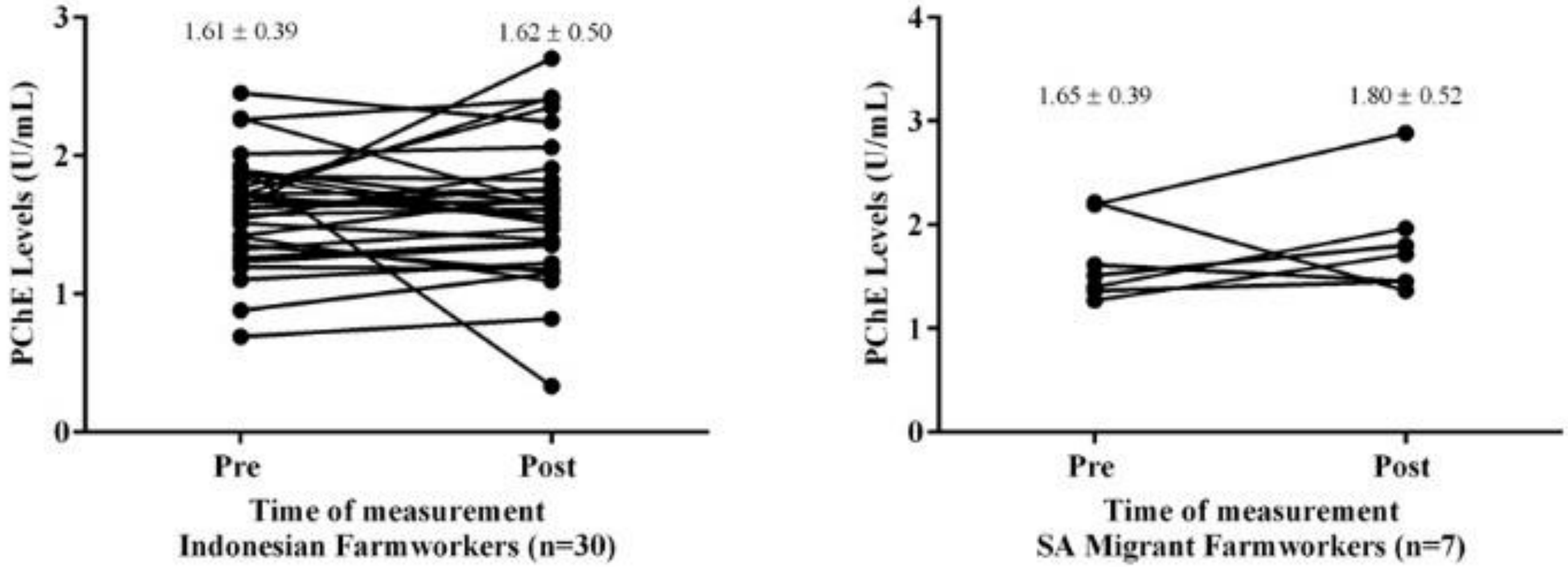

Figure 6: Activity levels of PChE in individually matched fingerprick blood samples in pre and post educational intervention among Indonesian ($p>0.05$) and SA migrant farmworkers ($p>0.05$).
Note: The data labels used in Figure 6 were mean ± SD.
Abbreviation: SD, standard deviation.

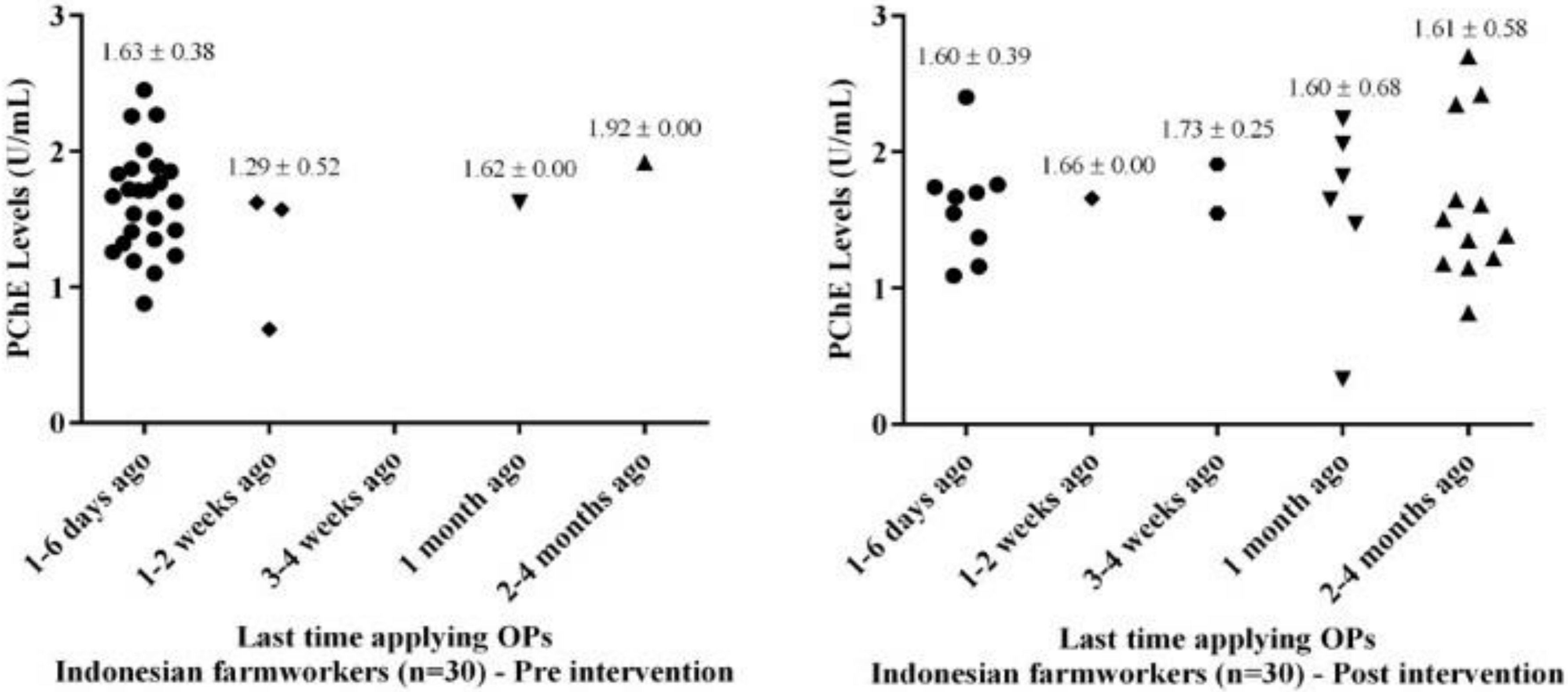

Figure 7: Last time applying OPs against PChE activity levels in pre and post educational intervention among Indonesian farmworkers.
Note: The data labels used in Figure 7 were mean ± SD.
Abbreviation: SD, standard deviation.

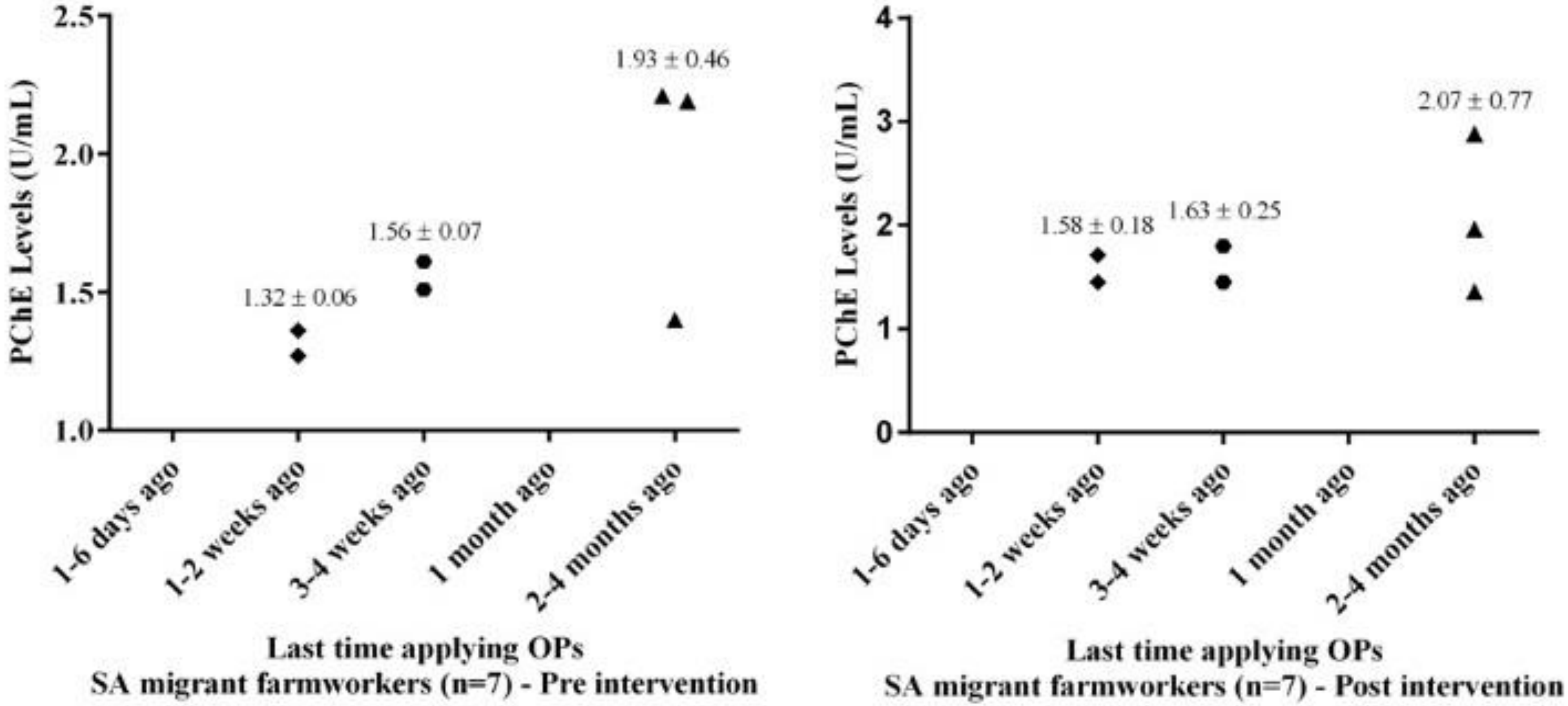

Figure 8: Last time applying OPs against PChE activity levels in pre and post educational intervention among SA migrant farmworkers.
Note: The data labels used in Figure 8 were mean ± SD.
Abbreviation: SD, standard deviation.

Figure 8 presents the last time applying OPs against PChE activity levels pre and post educational intervention among SA migrant farmworkers. In both pre and post intervention, mean PChE activity levels tended to increase among farmworkers who applied OPs more than 1-2 weeks ago, most clearly in the first set of measurements. The results of Spearman's Rank test showed that the last time applying OPs had statistically significant correlation with PChE activity levels in pre intervention ($p<0.05$), otherwise there was no statistically significant correlation in the post intervention ($p>0.05$).

3.4. Clinical Categories of EAChE and PChE Activity Levels

Table 2 presents clinical categories based on percentage of normal EAChE and PChE activity levels between Indonesian and SA migrant farmworkers. Nearly all farmworkers in both groups had normal levels of EAChE. At the same time, most of the research participants in both groups suffered from mild inhibition of PChE.

Table 2: Clinical categories based on percentage of normal EAChE and PChE activity levels among Indonesian and SA migrant farmworkers pre and post educational intervention.

Variable	**Indonesian farmworkers (n = 30)**		**SA migrant farmworkers (n = 7)**	
EAChE	Pre	Post	Pre	Post
Normal	29 (96.7%)	25 (83.3%)	6 (85.7%)	6 (85.7%)
Mild inhibition	1 (3.3%)	5 (16.7%)	1 (14.3%)	1 (14.3%)
Moderate inhibition	-	-	-	-
Severe inhibition	-	-	-	-
PChE				
Normal	5 (16.7%)	7 (23.4%)	2 (28.6%)	2 (28.6%)
Mild inhibition	24 (80.0%)	22 (73.3%)	5 (71.4%)	5 (71.4%)
Moderate inhibition	1 (3.3%)	1 (3.3%)	-	-
Severe inhibition	-	-	-	-

4. Discussion

This study found that mean EAChE activity levels post educational intervention were statistically different from the pre educational intervention among Indonesian farmworkers, compared with no significant change among SA migrant farmworkers (Figure 3). Generally, mean EAChE activity levels in both groups in the post intervention were lower than that in the pre intervention. Mean PChE activity levels in both groups did not significantly differ between the pre and post intervention measurements (Figure 6). The mean PChE levels in both groups in the post intervention were slightly higher than that in the pre intervention. Both mean EAChE (Figure 4 and Figure 5) and PChE (Figure 7 and Figure 8) activity levels tended to increase among farmworkers who applied OPs more than a month ago.

PChE inhibition is a biomarker of exposure to OPs, whereas inhibition of EAChE indicates a biomarker of toxicity and more indicative of the severity of poisoning (Lotti, 1995). PChE inhibition has been shown to not have relationship with EAChE inhibition (Lotti, 1995).

More than 80% of Indonesian farmworkers applied OPs within one to six days prior to the first measurement compared with about 29% of SA migrant farmworkers applying OPs within 1-2 weeks (Figure 1 and Figure 2). This contrasts with most of the research participants in both groups in the post intervention (40% and 43% respectively) estimating that they had applied OPs 2-4 months ago. Suratman et al. (2016, Chapter 3) reported there were statistically significant improvements in knowledge and perceptions of OP exposure after providing educational intervention among Indonesian and SA farmworkers involved in this study. According to Rogers (1983), improvement in knowledge as the first stage to adopting new ideas, playing an important role to change farmworkers' behaviour particularly in protecting themselves from OP exposure. However, when interpreting the effect of the educational intervention it is not clear whether the observed increase in PChE activity levels was a result of changed application behaviour or a result of a longer period between prior exposure and testing. From a practical point of view, possible differences between the groups might be related to the time elapsed since last exposure. Most of the SA migrant farmworkers applied OPs 1-2 weeks and 3-4 weeks before at the pre and post intervention. On the other hand, most of the Indonesian farmworkers applied OPs 1-6 days before at the pre-intervention, and only 30% at the post intervention time. However, Figure 4 and Figure 7 showed that mean EAChE and PChE activities among Indonesian farmworkers who applied OPs 1-6 days prior to data collection at the post intervention (25.89±5.11 U/g Hb and 1.60±0.39 U/mL respectively) were lower than that at the pre intervention (28.97±3.78 U/g Hb and 1.63±0.38 U/mL respectively). The Test Mate is useful for confirmation of a decrease in enzyme activity is suspected poisoning but it is not ideal for measuring more subtle changes (Rajapakse et al., 2011). Suratman et al. (2015, Chapter 4) reported the differences between Indonesian and SA migrant farmworkers in applying OPs compounds. All of Indonesian farmworkers sprayed OPs using backpack sprayer whereas a majority of SA migrant farmworkers sprayed OPs using hand spray gun. In addition, nearly all of Indonesian farmworkers used a tablespoon or a trowel to stir the mixture when mixing OPs without wearing gloves compared with SA migrant farmworkers that were better in handling OPs by wearing appropriate PPE. These differences in methods of OP application might be a major source of OP exposure particularly among Indonesian farmworkers. In addition, Suratman et al. (2015, Chapter 4) reported that most of Indonesian farmworkers who were entirely involved in this study in both measurements did not wear Personal Protective Equipment (PPE) like masks (83%), gloves (93%), and eye protection (97%) and sprayed OPs against the wind direction (60%), whereas most of SA migrant farmworkers involved in this study in both measurements wore appropriate PPE to reduce OP exposure, including chemical or waterproof boots (100%), mask (100%), gloves (71%), and eye protection (71%). The use of PPE plays an important role in the inhibition of EAChE and PChE (Jintana et al., 2009).

Indonesian farmworkers cultivated their crops and used OPs three times a year, February to May (rice), June to August (shallot), and November to January (shallot and chilli), whereas there was no cultivating activities from September to mid of November due to the very dry season. SA migrant farmworkers cultivated their crops during the periods of February to March, May to July, and August to November, with no cultivating activity from December to January. Interestingly, EAChE and PChE activity levels in one Indonesian farmworker dropped dramatically even though he reported applying OPs at least a month prior to the second measurement (Figure 3 and Figure 6). This might indicate unwitting OP exposure experienced by the farmworker. The risk of OP exposure is increased among agricultural workers resulting from unwittingly taken home OPs on clothing, shoes and other items (Ackerman & Cizmas, 2014). In contrast, EAChE and PChE levels in one of the SA migrant farmworkers declined substantially despite having applied OPs in the last 1-2 weeks prior to the second measurement. Immediately after exposure, PChE activity is more inhibited than EAChE (Lotti, 1995). The half-life of PChE recovery after OP exposure was about 12 days and complete recovery has been reported to occur after about 50 days (Mason, 2000). This contrasts with complete recovery of EAChE (attaining unexposed activity) after about 82 days, shorter than the normal life-span of erythrocyte (about 120 days) (Mason, 2000). Recovery from mild inhibition has been shown to be about 1-3 days whereas recovery from moderate inhibition is 1-2 weeks (Workplace Health and Safety Queensland, 2012).

Mean activity levels of EAChE and PChE in both groups in both measurements were lower than population references (31.4 U/g Hb for EAChE and 2.55 U/mL for PChE (EQM Research, 2011; Rajapakse et al., 2011). The population references were based on 40 normal male and female blood bank donor ranging from 20 to 60 years of age living in Midwestern United States (EQM Research, 2011). This results were similar to a study conducted by Ciesielski et al. (1994) in North Carolina in the United States that showed mean EAChE levels of 202 migrant farmworkers were 30.3 U/g Hb and approximately 12% of them had very low levels (below 25.3 U/g Hb). In this study, nearly all farmworkers in both groups had EAChE activity within the 'normal' range, when compared with the clinical guidelines for OP toxicity (Table 2). This contrasts with most Indonesian in the pre and post intervention suffered from mild inhibition of PChE activity levels (80% and 73% respectively) and about 3% of Indonesian farmworkers suffering from moderate inhibition of PChE activity. Most of SA migrant farmworkers remained constant, exhibiting mild inhibition in both time period of measurements (71%). These results are not consistent with a study conducted by Jintana et al. (2009) that indicated inhibition of PChE activity occurred in high-exposure period (3.73 ± 0.19 U/mL) compared to low-exposure period (4.92 ± 0.19 U/mL). Even though PChE inhibition is a biomarker of exposure to OPs, this parameter correlates very poorly with clinical signs or with EAChE inhibition (Eddleston et al., 2009).

EAChE measurement is a better predictor for effects compared with PChE measurement because EAChE found on erythrocyte membranes is similar to that found in neuronal tissue (Katz & Brooks, 2013; Mason, 2000; Office of Pesticide Programs, 2000).

There are several limitations in this study. The first being time since last exposure to OPs. This was reduced, but not eliminated, by selecting the research participants that had been working in farm field within the previous 3 months. Notwithstanding, the time between exposure varied between individuals and the estimates of time required each individual to remember when they last used OPs. The primary other limitation, discussed above, it that we do not know anything about other exposures, including being exposed to their own OPs by taking them home on clothes or exposure through food, etc.

5. Conclusions

Educational intervention provided to Indonesian and SA migrant farmworkers plays an important role in improving their knowledge and perceptions to reduce OP exposure. Mean EAChE activity levels in post educational intervention were statistically different from the pre educational intervention among the Indonesian farmworkers, but not among the SA migrant farmworkers. Mean PChE activity levels in Indonesian farmworkers slightly increased from 1.61 ± 0.39 U/mL in the pre-intervention to 1.62 ± 0.50 U/mL in the post-intervention. Similarly, Mean PChE activity levels in SA migrant farmworkers slightly rose from 1.65 ± 0.39 U/mL in the pre-intervention to 1.80 ± 0.52 U/mL in the post-intervention. However, mean PChE activities in both groups did not significantly differ between pre and post educational intervention. It is not clear whether the observed increase in PChE activity levels was a result of changed application behaviour or a result of a longer period between prior exposure and testing.

Both mean EAChE and mean PChE activity levels in both Indonesian and SA migrant farmworkers were lower than the population reference values. Based on percentage of normal EAChE activity, most of the research participants in both groups had normal levels. In contrast, most of farmworkers in both groups suffered from mild inhibition of PChE.

The inhibition of cholinesterase (ChE) activity levels after applying OPs compounds indicates exposure among farmworkers in both Indonesian and SA migrant farmworkers. OP application conducted by the research participants in both groups 1-6 days before collection and analysis of blood samples might increase possibility of ChE inhibition. Further research using a field study of PChE reactivator in both groups is warranted.

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Declarations of Interest

The authors declare that they have no conflicts of interest to report.

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Chapter 6. Pyridine-2-aldoxime methochloride as a PChE Reactivator

Estimation of Plasma Cholinesterase (PChE) inhibition using Pralidoxime (pyridine-2-aldoxime methochloride) as PChE reactivator in a field study

Suratman[1,2], **John William Edwards**[1], **Kateryna Babina**[1], **Kirstin Ross**[1]

1. Health and Environment Group, School of the Environment, Faculty of Science and Engineering, Flinders University, Adelaide, SA, Australia.
2. School of Public Health, Faculty of Health Sciences, Jenderal Soedirman University, Kampus Karangwangkal, Purwokerto 53122, Indonesia.

Keywords

Field study; fresh plasma blood; plasma cholinesterase reactivator; pyridine-2-aldoxime methochloride.

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Abstract

Estimating organophosphate pesticide (OP) exposure by measuring plasma cholinesterase (PChE) activities is confounded by not knowing the original, or unexposed activity. This study examined whether the addition of pralidoxime (pyridine-2-aldoxime methochloride) to blood samples would lead to measurable re-activation of plasma cholinesterase (PChE) activities in small volume fresh plasma blood samples to estimate the percentage inhibition of PChE activities due to OP exposure. This was an experimental study. Fingerprick blood samples were collected twice from 30 farmworkers at Dukuhlo Village in Brebes Regency, Indonesia and from seven South Australian (SA) migrant farmworkers. In addition, twenty-four venous blood samples from random blood donations were collected once from the Australian Red Cross Blood Service (ARCBS, Adelaide, SA). Blood samples were centrifuged to separate plasma. Plasma samples were then divided into two portions. One 8µL portion was mixed with 2µL pralidoxime solution in saline and the other portion was mixed with 2µL saline solution. PChE of each sample was analysed using the Test-mate ChE field kit. There were statistically significant differences between untreated plasma and treated plasma in PChE activities ($p<0.05$) for all groups. The increase of PChE activities in all analysed samples ranged from 36% - 39%. The estimation of percentage inhibition of PChE activity among these three groups showed that the highest inhibition occurred among SA migrant farmworkers, approximately 33%, while Indonesian farmworkers and ARCBS were similar, approximately 28%. This study demonstrated a simple and rapid method for estimating percentage PChE inhibition in a single blood sample.

1. Introduction

Organophosphate pesticides (OPs) are inhibitors of cholinesterase (Marrs, 2001). Farmworkers around the world use OPs to reduce the impact of pests on their crops. Suratman et al. (2015a, Chapter 1) demonstrated that OP poisoning in farmworkers is a major public health problem in developing and developed countries (Afriyanto, 2008; Beseler & Stallones, 2008; Das et al., 2002; Dasgupta et al., 2007; Faria et al., 2014; He, 1996; Jeyaratnam, 1990; Kir et al., 2013; Kishi et al., 1995; Lee et al., 2011; Murali et al., 2009; Peshin et al., 2014; Rajashekhara et al., 2013; Rustia et al., 2010; X. Zhang et al., 2011; Zilker, 1996). Suratman et al. (*submitted*, Chapter 5) reported that approximately 80% of Indonesian farmworkers suffered from mild inhibition of plasma cholinesterase (PChE) activities and 3% suffered from moderate inhibition. This compares with approximately 70% of South Australian migrant farmworkers suffering from mild inhibition and 0% from moderate inhibition of PChE activities due to OP exposure. Some causal agents of pesticide poisonings as reported in the literature are chlorpyrifos, diazinon, and malathion, which are the most common OPs used by farmworkers (Weiss et al., 2004; WHO, 2009).

OPs inhibit the activity of cholinesterase (ChE), an enzyme responsible for the hydrolysis of acetylcholine (ACh), an excitatory neurotransmitter, into choline and acetate in order to prevent over-stimulating post-synaptic nerves, muscles, and exocrine glands (Heide, 2007; Marrs, 2001). Failure of ChE hydrolysis results in the excessive accumulation of of ACh in the synaptic cleft (Karalliedde & Henry, 2001; Marrs, 2001). Specific cholinesterases, known as erythrocyte acetylcholinesterase (EAChE), are found in the nerve ganglion synapses and erythrocytes, whereas non-specific cholinesterases, known as butyrylcholinesterase (BuChE) and plasma cholinesterase (PChE), are mainly found in plasma and liver (Marrs, 2001). OP exposure leads to the inhibition of ChE via the reduction of the alkyl group and results in irreversible inactivation of the enzyme, a process known as ‘enzyme ageing’ (Jokanovic & Prostran, 2009; Mercey et al., 2012; South Asian Cochrane Network and Centre (SASIANCC), 2012; Worek et al., 2005). Once inhibited by OP binding, ChE can undergo one of two fates, either spontaneous reactivation or ageing to a permanently inhibited structure. Dimethyl OPs such as azinphos-methyl and phosmet cause ChE ageing much faster than diethyl OPs such as chlorpyrifos and diazinon (Eddleston et al., 2008). Chlorpyrifos was the common active constituent of OPs used by Indonesian farmworkers and SA migrant farmworkers (50% and 43% respectively) (Suratman et al., 2015b, Chapter 4).

Anticholinergic drugs (atropine), anticonvulsants (diazepam), and oximes had been used to treat OP poisoning depending on chemical structures and type of OPs inhibitor (Jun et al., 2011). Pralidoxime (pyridine-2-aldoxime methochloride) is an oxime, that has been widely used to reactivate OPs-inhibited human EAChE and PChE (Eddleston et al., 2009; Jun et al., 2011; Kuca et al., 2010; Rajapakse et al., 2011; Worek et al., 1997; Y. H. Zhang et al., 2007). Pralidoxime is an OP-specific selective ChE re-activator (Costa et al., 2011; Mahesh et al., 2013).

PChE inhibition is used as a biomarker of exposure to OPs and is used to monitor farmworkers at risk of OP exposure (Lotti, 1995; Mason, 2000). Unless both pre-exposure and post-exposure measurements of PChE are compared, it is not clear what an individual’s pre-exposure PChE levels might be. It is often impractical to perform both pre- and post-exposure measurements, especially under field conditions. Using PChE re-activator in the analysis of post-exposure samples may provide an alternative to having to collect and analyze two blood samples, as well as improving the sensitivity of the ChE test.

The aims of this study were to examine whether the addition of pralidoxime to the blood samples would lead to measurable re-activation of PChE activities in small volume fresh plasma samples in the field as a method to estimate percentage inhibition of PChE activities due to OP exposure.

2. Study Design and Methods

This was a true experimental study (Campbell & Stanley, 1966). Fingerprick blood samples were collected twice from thirty farmworkers working on conventional farms at Dukuhlo Village in Brebes Regency, Central Java, Indonesia in July-August 2014 and in November-December 2014 and from seven South Australian (SA) migrant farmworkers (Vietnamese) from Virginia in South Australia in May-June 2014 and in September-October 2014. In addition, twenty-four venous blood samples were collected once at the Australian Red Cross Blood Service (ARCBS), Adelaide, South Australia in February-March 2015.

Figure 1 presents the sample analysis process for each participant.

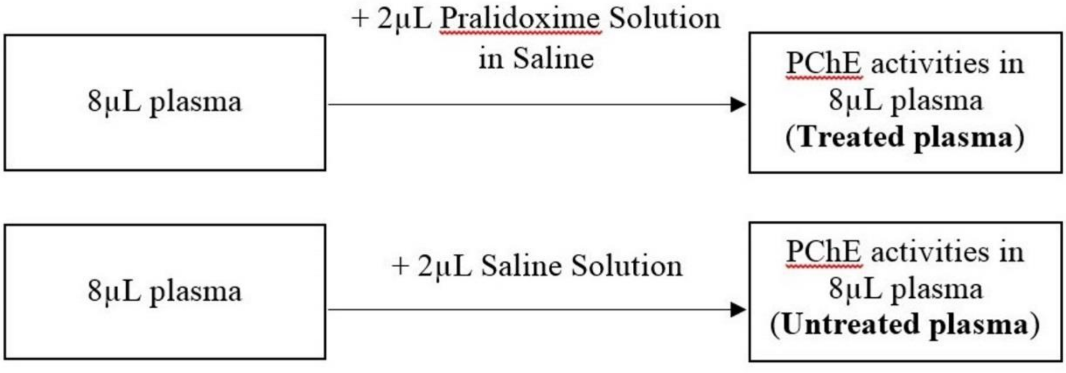

Figure 1: The sample analysis process for each participant.

Ethics approvals were obtained from Southern Adelaide Clinical Human Research Ethics Committee (SACHREC) with approval number: 319.13, Commission on Health Research Ethics, Faculty of Public Health, Diponegoro

University, Semarang, Indonesia with approval number: 183/EC/FKM/2013, and from the ARCBS with approval number: N15-02SA-03.

2.1. Procedures

Preparation of saline solution and pralidoxime solution in saline:

1) Saline solution

Saline solution was prepared according to manufacturer's instructions (Sigma-Aldrich Co., Ltd., USA). Briefly, one tablet of Phosphate Buffered Saline was dissolved in 200 mL distilled water yielding 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, and sterilised by autoclaving at 165°C for 30 min. Saline solution was allowed to cool to about 25°C prior to use.

2) Pralidoxime solution in saline

To make stock concentration of pralidoxime solution in saline, 431.55mg of pralidoxime was dissolved in 5 mL saline solution. Each experiment was conducted by taking 2µL pralidoxime solution in saline, then adding to 8µL plasma resulting in a final concentration of 100 µmol/l. This final concentration referred to the result of a randomised control trial of pralidoxime in acute OP insecticide poisoning conducted by Eddleston et al. (2009) that found a steady state plasma pralidoxime concentration was around 100 µmol/l. In addition, concentration of pralidoxime that had a high in vitro effect on human cholinesterase was at approximately 100 µmol/l (Eyer, 2003).

2.2. Sample Collection

All blood samples were centrifuged to separate plasma from erythrocytes. Plasma was then divided into two portions. One 8µL portion was mixed with 2µL pralidoxime solution in saline as treated plasma and the other 8µL portion was mixed with 2µL pure saline solution as untreated plasma. All tests used 10µL capillary tubes. PChE activities were measured using the Test-mate ChE Cholinesterase System Test field kit (EQM Research Inc., Cincinnati). The entire assay of each sample was completed in approximately 4 minutes and the temperature ranged from 22°C to 30°C. The recommended temperature range for the assay procedure is between 15°C to 30°C (EQM Research, 2011).

2.3. Data Analysis

Statistical analyses were performed using the statistical package SPSS version 17 (SPSS Inc., Chicago, IL, USA). Graphs were created using GraphPad Prism v6.05 (GraphPad Software Inc., 2014). Continuous data were tested for normality using the Shapiro-Wilk test (Elliot & Woodward, 2007; Razali & Wah, 2011). If a normal distribution was found, data were expressed as means and standard deviations and were analysed using parametric methods (a paired t test, an unpaired t test, and Analysis of Variance (ANOVA)). Otherwise data were expressed as medians and ranges and analysed using non-parametric methods (Wilcoxon test, Mann-Whitney U test, and Kruskal-Wallis test). Level of statistical significance was set at $\alpha = 0.05$.

The percentage of PChE inhibition (% INH) was calculated from the following equation:

$$\%INH = \left[\frac{(PChE\ levels\ in\ pralidoxime\ with\ saline - PChE\ levels\ in\ saline)}{PChE\ levels\ in\ pralidoxime\ with\ saline}\right] x\ 100$$

where *% INH* is the percentage inhibition of PChE, *PChE levels in pralidoxime with saline* is the levels of PChE measured in 10μL capillary tube consists of 8μL fresh plasma and 2μL pralidoxime solution in saline, called as *treated plasma*, and *PChE levels in saline* is the levels of PChE measured in 10μL capillary tube consists of 8μL fresh plasma and 2μL saline solution, called as *untreated plasma*.

The results of PChE levels in pralidoxime with saline measurements were adjusted by a blank (minus plasma samples) to correct for the contribution of pralidoxime solution to absorbance on each day.

3. Results

Figure 2 presents PChE activities in individually matched plasma samples collected from 30 Indonesian farmworkers, seven SA migrant farmworkers, and 24 blood bank samples between untreated and treated plasma.

Results from Indonesian farmworkers found that in the first sampling measurement (Figure 2-A), the mean PChE activity in untreated plasma was 2.07U/mL whereas the mean PChE activity in treated plasma was 2.87U/mL (increase of approximately 39%). Paired *t* test analyses showed that there was a statistically significant difference between untreated and untreated plasma for the PChE activities ($p<0.05$). Similarly, in second sampling measurement (Figure 2-B), the mean PChE activity in untreated plasma was 2.09U/mL whereas the mean PChE activity in treated plasma was 2.84U/mL (increase by about 36%). There was a statistically significant difference between untreated and treated plasma for the PChE activities ($p<0.05$).

Results from SA migrant farmworkers showed that in first sampling measurement (Figure 2-C), the mean PChE activity of untreated plasma was

2.08U/mL whereas the mean PChE activity of treated plasma was 2.83U/mL (increased about 36%). Paired *t* tests showed that there was a statistically significant difference between untreated and treated plasma for the PChE activities ($p<0.05$). Similarly, in second sampling event (Figure 2-D), the mean PChE activity in untreated plasma was 2.02U/mL whereas the mean PChE activity in treated plasma was 2.80U/mL (rose approximately 39%). There was also a statistically significant mean difference between untreated and treated plasma for the PChE activities ($p<0.05$).

Blood samples collected once from ARCBS showed that the mean PChE activity in untreated plasma was 2.13U/mL whereas the mean PChE activity in treated plasma was 2.93U/mL (increased approximately 38%) (Figure 2-E). The result of paired t test showed that there was statistically significant difference between untreated and treated for the PChE activities ($p<0.05$).

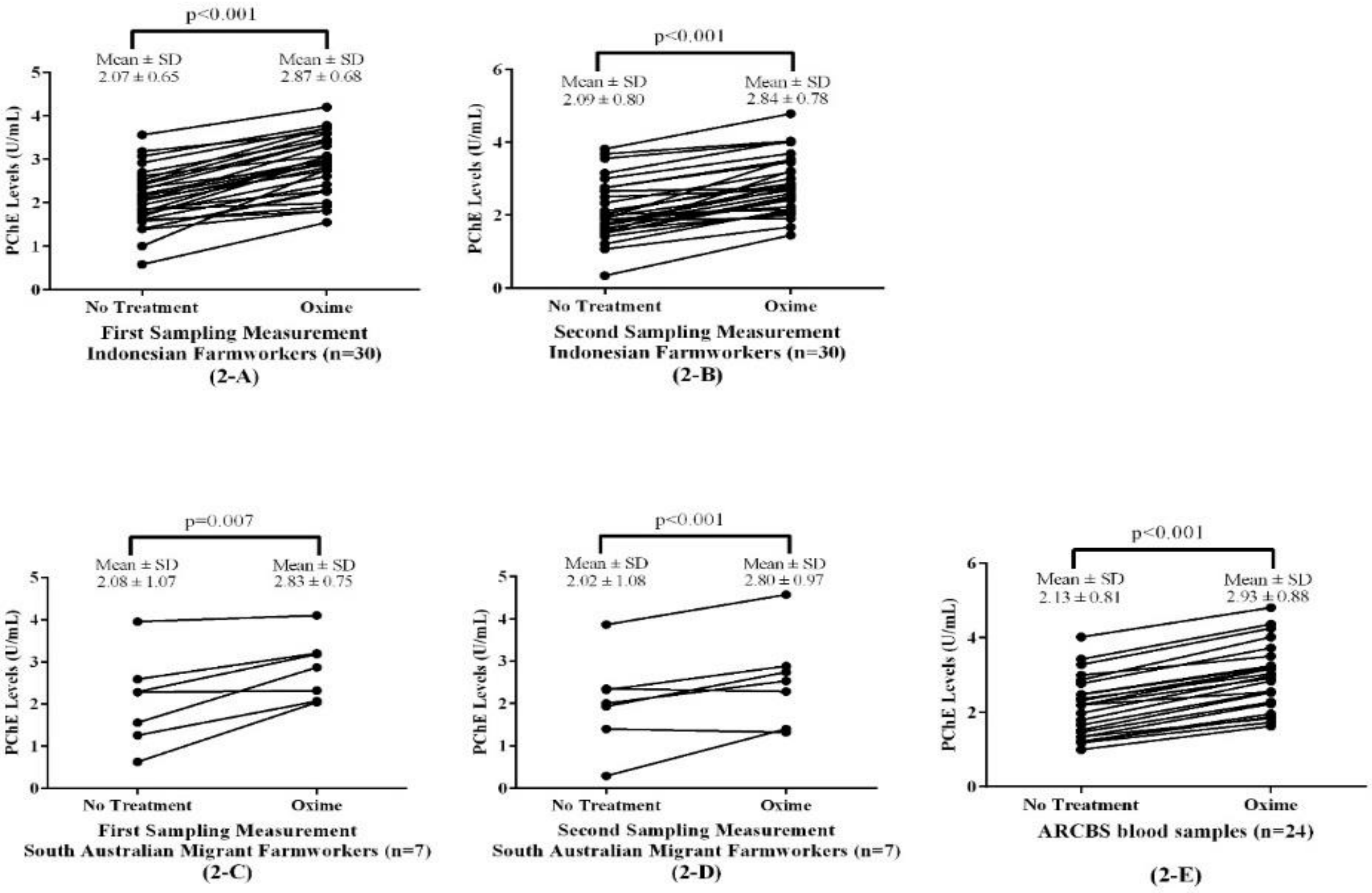

Figure 2: PChE activities in individually matched plasma samples obtained from Indonesian farmworkers, SA migrant farmworkers, and ARCBS between untreated and treated plasma.

Figure 3 presents differences in measured percentage PChE Inhibition (%INH) between Indonesian farmworkers, SA migrant farmworkers, and blood bank samples (ARCBS).

The percentage PChE inhibition among Indonesian farmworkers was approximately 28%, was quite similar to the percentage of PChE inhibition among ARCBS. On the other hand, the estimation of percentage of PChE inhibition among SA migrant farmworkers was slightly higher than that of these two groups, approximately 30%.

Analysis of variance indicated that there were no statistically significant differences in percentage of PChE inhibition between Indonesian farmworkers, SA migrant farmworkers, and ARCBS (p>0.05).

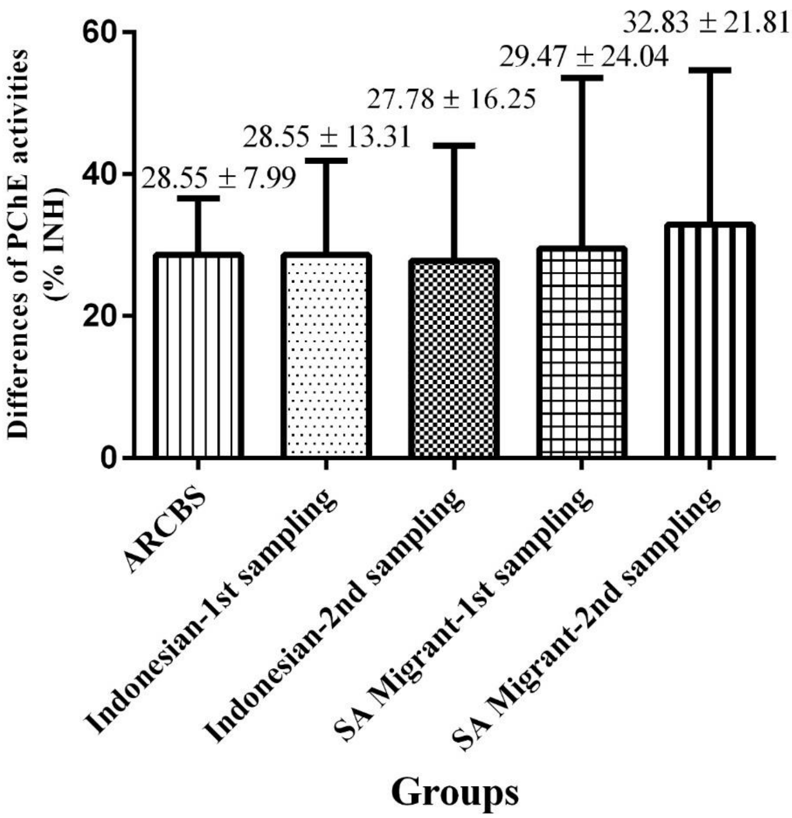

Figure 3: Differences in estimation of a percentage of PChE inhibition (% INH) between Indonesian farmworkers, SA migrant farmworkers, and ARCBS.

4. Discussion

This study found activities of cholinesterase (ChE) in 8μL fresh plasma blood samples inhibited by OPs significantly increased after adding 2μL pralidoxime solution in saline in all samples from the three study groups (Indonesian farmworkers, SA migrant farmworkers, and ARCBS).

Pralidoxime is one of therapeutic agents commonly used in the medical treatment and commercially available to reactivate ChE due to OPs (Eddleston et al., 2008). Pralidoxime reversibly binds to ChE molecule at a catalytic site (active centre), allosteric (peripheral), or at both sites, then performs nucleophilic attack at the phosphorus atom of the OPs residuum by which it yields the formation of an unstable enzyme-inhibitor-oxime complex and splits the complex into a phosphorylated oxime and reactivated enzyme (Stojiljkovic & Jokanovic, 2006).

Pralidoxime-induced reactivation of PChE activity inhibited by OPs ranged from 36% to 39% in our study population (Figure 2). This is higher reactivation of PChE activities than was previously reported by Jun et al. (2011), who reported that PChE reactivation ability of pralidoxime was less than 6%. On the other hand, our results agree with the study published by Kuca et al. (2010) who found ChE activity levels inhibited by chlorpyrifos significantly increased about 80% after providing pralidoxime as a ChE reactivator using in vitro method. The difference in PChE activities between treated and untreated plasma samples may reflect the presence of quite recently inhibited ChE. A study by Suratman et al. (*submitted*, Chapter 5) among Indonesian and SA migrant farmworkers who were also being the research participants in this study reported that more than 80% of Indonesian farmworkers applied OPs within one to six days prior to the first measurement compared with about 29% of SA migrant farmworkers applying OPs within 1-2 weeks. This results indicated recent OP exposure occurred among farmworkers in both study groups. On the other hand, most of the research participants in both groups in the second measurement (40% and 43% respectively) estimating that they had applied OPs 2-4 months ago. A study by Konickx et al. (2013) showed that butyrylcholinesterase (BuChE) regeneration with pralidoxime treatment was small after 2 diethyl OP insecticides intoxication, such as chlorpyrifos and quinalphos, otherwise it was non-existent with the dimethyl OPs, dimethoate or fenthion. In other words, the degree of butyrylcholinesterase (BuChE) reactivation would depend on the OP to which the worker was exposed to. This might influence the results in the field.

As a biomarker of exposure, PChE levels are known to be associated with seasonal sprayer activity (Roldan-Tapia et al., 2006). According to Mason (2000), PChE activities inhibited by OPs has a half-life of recovery around 12 days and a complete recovery after about 50 days.

The estimation of percentage inhibition of PChE activity in fresh plasma samples due to OP exposure among these three groups showed that the highest inhibition occurred among SA migrant farmworkers, approximately 33%, while Indonesian farmworkers and ARCBS were similar, approximately 28% (Figure 3). The pattern of farming methods might have an important role in distinguishing percentage inhibition of PChE activities among Indonesian farmworkers and SA migrant farmworkers except for ARCBS. Indonesian farmworkers cultivated their crops using a method of open farm whereas SA migrant farmworkers planted their crops in greenhouse. These differences in worksite conditions might play an important role in modulating exposure to OPs.

Interestingly, the estimation of percentage inhibition of PChE activity in fresh plasma samples in blood bank (ARCBS) is quite similar to the result shown in Indonesian farmworkers (Figure 3). This result may indicate that there is any OP exposure among those who donated blood. Unfortunately, there is no information obtained from ARCBS about their personal identities including age and types of works due to confidentiality.

This study has limitations as follows: 1) test of validity for pralidoxime effects was not conducted using the Australian blood bank samples in a conventional laboratory assay; 2) changing concentration of pralidoxime to establish that this was an effect related to pralidoxime treatment was not conducted to confirm the effect.

5. Conclusions

The addition of 2µL pralidoxime solution in saline significantly leads to measurable re-activation of PChE activities in 8µL fresh plasma samples in the field. The increase of PChE activities in all analysed fresh plasma samples ranged from 36% - 39% after being reactivated by pralidoxime solution in saline.

The results of re-activation of PChE activities in this study can be used to estimate percentage inhibition of PChE activities due to OP exposure based on the PChE re-activation after the samples had been treated with pyridine-2-aldoxime methochloride. This study demonstrated a simple and rapid method for estimating percentage PChE inhibition in a single blood sample. The method described in this study uses small volume blood samples and potable equipment, which makes it valuable for rapid field based testing in occupational groups.

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Declarations of Interest

The authors declare that they have no conflicts of interest to report.

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Chapter 7. Final Discussion

Research examining factors contributing to organophosphate pesticide (OP) exposure among farmworkers by comparing developed countries and developing countries is limited (Suratman et al., 2015a, Chapter 1). Few studies have compared biological monitoring such as levels of cholinesterase in blood and metabolites in urine samples with adverse health effects due to pesticide exposure associated with risk factors (Suratman et al., 2015a, Chapter 1). To date, most studies have examined only risk factors of pesticide exposure without direct biomarker measurements (Suratman et al., 2015a, Chapter 1).

OPs have been used in farming worldwide. OPs contribute to mortality and morbidity in farmworkers all around the world (Jeyaratnam, 1990). Morbidity and mortality rates globally have increased due to the increase of acute pesticide poisoning cases (Jeyaratnam, 1990; Kishi & LaDou, 2001). Globally, World Health Organisation (WHO) estimates three million cases of pesticide poisoning occur every year, yielding in an excess of 250,000 deaths (WHO, 2006). Adverse health effects due to OP exposure in developing (Afriyanto, 2008; Dasgupta et al., 2007; Faria et al., 2014; He, 1996; Jeyaratnam, 1990; Kir et al., 2013; Kishi et al., 1995; Murali et al., 2009; Peshin et al., 2014; Rajashekhara et al., 2013; Rustia et al., 2010; Zhang et al., 2011) and developed countries (Beseler & Stallones, 2008; Das et al., 2002; Jeyaratnam, 1990; Lee et al., 2011; Zilker, 1996) are a major public health problem. Farmworkers are at risk of exposure to OPs. However, few studies have been conducted that aim to improve farmers' knowledge and perceptions of OP exposure using pesticide safety training associated with biomarker assessment. Afriyanto (2008) identified the need for providing pesticide safety interventions to improve knowledge and perceptions of OP exposure to reduce the risk of OP poisonings among farmworkers in Indonesia. Similarly, Johnstone (2006) found the need for refreshing knowledge and improving awareness in safety among farmworkers in Australia.

Research presented here and published in peer reviewed journals as an outcome of this study investigated behavioural risk factors for OP exposure using the Health Belief Model (HBM) theory, biological monitoring of effects, and the impact of an intervention to reduce exposure among Indonesian and South Australian (SA) migrant farmworkers. According to Arcury et al. (2002), the HBM theory is a suitable model to study farmworker pesticide safety behaviour due to its simplicity and parsimony. The HBM has four perceptions serve as the main constructs of the model as follows: perceived susceptibility, perceived severity, perceived benefits, perceived barriers which are modified by other variables such as culture, education level, age, gender, ethnicity, past experience, knowledge, and cues to action (Champion & Skinner, 2008; Stretcher et al., 1997).

The factors of knowledge (knowledge about adverse effects of OPs and knowledge about self-protection from OP exposure) and perceptions of OP exposure (perceived susceptibility, perceived severity, perceived benefits, perceived barriers, and cues to action) in the pre and post educational intervention were assessed (Suratman et al., 2016, Chapter 3). Generally, Indonesian farmworkers had better knowledge and perceptions than SA migrant farmworkers after adjusting the variables of years working as a farmworker and level of education. Years working as a farmworker, called as work experience, has direct relationship with knowledge and perceptions (Tesluk & Jacobs, 1998). Meanwhile, level of education is often linked to how people behave when facing risks (UNEP, 2005). Having at least a high school education influenced the perceptions of farmworkers to usefulness of PPE (Hwang et al., 2000). In addition, the effectiveness of providing pesticide safety education as an intervention to improve knowledge and perceptions of OP exposure to reduce OP exposure was assessed (Suratman et al., 2016, Chapter 3). The method of the intervention provided to Indonesian farmworkers used a group communication approach, compared with the intervention for SA migrant farmworkers, which used an individual communication approach. Indonesian farmworkers had statistically significant improvements in most aspects of knowledge and perceptions about OP exposure in the follow-up measurement after providing the interventions. Conversely, SA migrant farmworkers had not statistically insignificant improvements in all measured variables, except for knowledge about adverse effects of OPs (Suratman et al., 2016, Chapter 3). This might have been due to the greater knowledge already held by the SA migrant farmworkers. Alternatively, this might be due to the different methods of the interventions provided to both groups. The use of group communication was more effective to improve farmworkers' knowledge and perceptions than individual approach. Different methods of educational interventions between groups might influence effectiveness of provided interventions (ILEP, 1998). Field practices in handling OPs and OP-related symptoms in pre and post intervention were assessed in Chapter 4 (Suratman et al., 2015b, Chapter 4). Generally, SA migrant farmworkers had better field practices in handling OPs than Indonesian farmworkers in pre educational intervention. Notwithstanding, some significant behavioural improvements in handling OPs occurred among Indonesian farmworkers as the result of the intervention (Suratman et al., 2015b, Chapter 4). On the other hand, the field practices of SA migrant farmworkers in post educational intervention remained constant (pre intervention). According to the HBM theory, perceived susceptibility is a strong predictor of preventive health behaviour (Champion & Skinner, 2008). Perceived susceptibility refers to person's subjective perceptions regarding the risk of health conditions. In the case of a medical illness, these dimensions include acceptance of a diagnosis, personalized forecast for the re-susceptibility and susceptibility towards a disease in general (Brandt et al., 2001; Champion & Skinner, 2008; Clark & Houle, 2009). Feeling susceptible to a condition which leads to a serious disease can encourage farmworkers to change their behaviour (Champion & Skinner, 2008; Stretcher et al., 1997). It depends on one's belief of the effectiveness of the various measures available to reduce the threat of disease, or the perceived benefits in making health efforts. Meanwhile, perceived severity refers to feelings about the seriousness of the disease, including the evaluation of the clinical and medical consequences (e.g. death, disability, and pain) and social consequences that may occur (such as the effects on employment, family life and social relationships) (Brandt et al., 2001; Champion & Skinner, 2008; Clark & Houle, 2009). Perceived barriers appear due to heightened view of potential negative aspects of health-related behaviour change. Factors, such as uncertainty, side effects, questions about suitability, anxiety and stress may act as a barrier to change behaviour (Brandt et al., 2001; Champion & Skinner, 2008; Clark & Houle, 2009). In addition, behaviour is also influenced by cues to action. Cues to action are events, things or people that encourage or trigger people to change their behaviour by using appropriate reminder systems, promoting awareness, or providing information (Brandt et al., 2001; Champion & Skinner, 2008; Clark & Houle, 2009).

These relationships were detected despite data limitations, which included small sample population limited to one village in Indonesia (30 Indonesian farmworkers) and one region in Australia (7 SA migrant farmworkers). The implications of these significant findings are that Indonesian farmworkers are at higher risk than SA migrant farmworkers in both their exposure to OPs and their level of adverse health effects. These results may reflect the differences of law enforcement between both countries. Australian government has strictly regulated the use of chemicals, including pesticides, at both federal and state levels. All farmworkers and pesticide applicators in South Australia are expected to adhere to legislation. Approximately 78% of farmworkers had completed the ChemCert course for chemical accreditation (Johnstone, 2006). Federally, the Australian Pesticides and Veterinary Medicines Authority (APVMA) plays an important role and has responsible for the evaluation, registration and review of agricultural and veterinary chemicals, and their control up to the point of retail sale (ChemCert Australia, 2013). In South Australia, the major legislation available relating to agricultural and veterinary chemical use is: the Agricultural and Veterinary Products (Control of Use) Act 2002; the Agricultural and Veterinary Products (Control of Use) Regulations 2004; the Livestock Act 1997; the Controlled Substances Act 1984; SA Occupational Health, Safety and Welfare Act 1986; and OHSW Regulation 1995 (ChemCert Australia, 2013).

In Indonesia, there are some regulations relating to chemicals and pesticides and to prevent adverse health effects released by President of Republic of Indonesia, Minister of Health, and Minister of Agriculture. Legislation published by President of Republic of Indonesia is as follows: 1) Law No. 10/2013 on Rotterdam Convention on The Prior Informed Consent Procedure For Certain Hazardous Chemicals and Pesticides in International Trade (*Konvensi Rotterdam tentang Prosedur Persetujuan Atas Dasar Informasi Awal untuk Bahan Kimia dan Pestisida Berbahaya Tertentu dalam Perdagangan Internasional*); and 2) Law No. 36/2009 on Health (*Undang-undang No. 36 Tahun 2009 tentang Kesehatan*). Legislation published by Minister of Health was as follows: 1) Regulations of Minister of Health No. 258/MENKES/PER/III/1992 on Health Requirements of Pesticide Management (*Persyaratan Kesehatan Pengelolaan Pestisida*); 2) Regulations of Minister of Health No. 374/MENKES/PER/III/2010 on Vector Control (*Pengendalian Vektor*).

Legislation published by Minister of Agriculture was as follows: 1) Regulations of Minister of Agriculture No. 107/Permentan/SR.140/9/2014 on Pesticide Monitoring (*Pengawasan Pestisida*); 2) Regulations of Minister of Agriculture No. 01/Permentan/OT.140/1/2007 on Lists of Active Constituents of Banned and Restricted Pesticides (*Daftar Bahan Aktif Pestisida yang Dilarang dan Pestisida Terbatas*); and 3) Regulations of Minister of Agriculture No. 24/Permentan/SR.140/4/2011 on Requirements and Procedures of Registering Pesticide (*Syarat dan Tatacara Pendaftaran Pestisida*). However, the awareness of Indonesian farmworkers to prevent OP exposure was low, which was reflected by not wearing appropriate Personal Protective Equipment during working with OP compounds (Suratman et al., 2015b, Chapter 4). Consequently, law enforcement of these regulations in Indonesia might need to be improved in order to be strictly obeyed by farmworkers.

Biological monitoring of effects to measure levels of erythrocyte acetylcholinesterase (EAChE) and plasma cholinesterase (PChE) activities in whole blood samples was presented in Chapter 5 (Suratman et al., *submitted-b*, Chapter 5). Generally, mean EAChE activities in both groups in the post educational intervention were lower than that of in the pre educational intervention. Meanwhile, mean PChE activities in both groups did not significantly differ between before and after the educational intervention. Both mean EAChE and PChE activities in both Indonesian and SA migrant farmworkers were lower than the population reference values. Indonesian farmworkers cultivated their crops and used OPs three times a year, namely February to May (rice), June to August (shallot), and mid of November to January (shallot and chilli) whereas there was no cultivating activities from September to mid of November due to very dry season. SA migrant farmworkers cultivated their crops during the periods of February – March, May – Mid of July, and August – November whereas there was no cultivating activity from December of January. Interestingly, individually, mean EAChE and PChE levels in one of the Indonesian farmworkers dropped dramatically even though he applied OPs in the previous month in the second measurement. This might indicate unwitting OP exposure experienced by the farmworker. The risk of OP exposure increases among agricultural workers due to OPs that are unwittingly taken home on clothing, shoes and other items (Ackerman & Cizmas, 2014). Based on percentage of normal EAChE activity, most of the research participants in both groups had normal levels. In contrast, based on percentage of normal PChE activity, more than 70% farmworkers in both groups suffered from mild inhibition. These findings, particularly for among Indonesian farmworkers, were consistent with other studies conducted in Indonesia by Rustia et al. (2010) that indicated 71.4% of 56 pesticide applicators in Tanggamus Regency, Lampung Province suffered from mild inhibition and 28.6% of them suffered from moderate inhibition due to OP exposure. Even though PChE inhibition is a biomarker of exposure to OPs, this parameter correlates very poorly with clinical signs or with EAChE inhibition (Eddleston et al., 2009).

Due to many cases of PChE inhibition among farmworkers, reactivation ability of pyridine-2-aldoxime methochloride as a field study PChE reactivator to estimate percent inhibition of PChE (% INH) using fresh plasma blood samples was examined in Chapter 6 (Suratman et al., *submitted-a*, Chapter 6). This study found mean PChE activities increased ranging from 36% to 39% after providing 2 µl pralidoxime in saline solution in all plasma blood samples (Suratman et al., *submitted-a*, Chapter 6). Percent inhibition of PChE among SA migrant farmworkers was slightly higher than that of the other two groups. In comparison with Indonesian farmworkers who cultivated their crops using a method of open farm, SA migrant farmworkers cultivated their crops in greenhouse. The types of worksites might contribute to increase absorption of OP compounds among SA migrant farmworkers rather than among Indonesian farmworkers.

Overall, the findings of this study reflect the differences in all aspects relating to OP exposure in both countries. Most of Indonesian farmworkers were untrained in handling OPs compared with SA migrant farmworkers. Low level of education, low knowledge, and poor perceptions were the most likely factors contributing to unsafe work practices commonly found in their workplace that increased OP exposure among them. Indonesian farmworkers had significant improvement in their knowledge and perceptions of OP exposure after being provided with the educational intervention, although theoretically they need long time to change their work practices in order to be safer and healthier. Long-term educational intervention is needed in order to change work practices of farmworkers in both Indonesian and SA migrant farmworkers in handling OPs and to reduce OP exposure. In addition, further study of the other intrinsic factor increasing susceptibility to OP effects like paraoxonase (PON1) that plays an important role the pathogenesis of atherosclerosis and is primarily associated with the hydrolysis of OP compounds need to be conducted to measure chronic exposure to OPs associated with the decrease of PON1 activity.

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Appendices

Appendix 1

Calculation of Sample Size using STATA IC version 12.1 software (Copyright 1985-2011 StataCorp LP)

Sample size calculation for test of means with repeated measures

. sampsi 1.5 2.0, sd1(0.3) sd2(0.4) alpha(0.05) pre(1) post(1) r01(0.2) method(change)

Estimated sample size for two samples with repeated measures
Assumptions:

alpha	=	0.0500 (two-sided)
power	=	0.9000
m1	=	1.5
m2	=	2.0
sd1	=	0.3
sd2	=	0.4
n2/n1	=	1.0
number of follow-up measurements	=	1
number of baseline measurements	=	1
correlation between baseline & follow-up	=	0.200

Method: CHANGE (difference between follow-up and baseline mean)

relative efficiency	=	0.625
adjustment to sd	=	1.265
adjusted sd1	=	0.379
adjusted sd2	=	0.506

Estimated required sample sizes:
n1 = n2 = 20

Remarks:

Level of significance	= 0.05
Power of the test	= 90%
Mean of PChE level (30% - 74% of normal) based on previous research (Miranda-Contreras et al., 2013)	= 1.5 U/mL
Standard Deviation (SD) of PChE level (30% - 74% of normal) based on previous research (Miranda-Contreras et al., 2013)	= 0.3 U/mL
Mean of normal PChE level in population (EQM Research, 2011)	= 2.0 U/mL
SD of normal PChE level in population (EQM Research, 2011)	= 0.4 U/mL
Sample size of Indonesian farmworkers (n1)	= 20
Sample size of South Australian migrant farmworkers (n2)	= 20

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Appendix 2

Consent to participation in research

I, ...

....

(first or given names) (last name)

give consent to my involvement in the research project (short title):

Risk Factors for Organophosphate Pesticide (OP) Exposure among Indonesian and South Australian Migrant Farmworkers and the Impact of an Intervention to Reduce Exposure

I acknowledge the nature, purpose and contemplated effects of the research project, especially as far as they affect me, have been fully explained to my satisfaction by

....

....

(first or given names) (last name)

and my consent is given voluntarily.

I acknowledge that the detail(s) of the following has/have been explained to me, including indications of risks; any discomfort involved; anticipation of length of time; and the frequency with which they will be performed:

1. Complete questionnaire on personal factors and work practices on 2 occasions
2. Provide a fingerprick blood sample on 2 occasions.
3. Participate in an information/training session on 1 occasions

I have understood and am satisfied with the explanations that I have been given.

I have been provided with a written information sheet.

I understand that my involvement in this research project may not be of any direct benefit to me and that I may withdraw my consent at any stage without affecting my rights or the responsibilities of the researchers in any respect.

I declare that I am over the age of 18 years.

I acknowledge that I have been informed that should I receive an injury as a result of taking part in this study, I may need to start legal action to determine whether I should be paid.

Signature of Research Participant: ……………………………………………………….

Date: ....

I, ……………………………….. have described to ………………………………….

the research project and nature and effects of procedure(s) involved. In my opinion he/she understands the explanation and has freely given his/her consent.

Signature: ....

Date: ....

Status in Project: ……………………………………………………

Appendix 3

Participant Information Sheet

Risk Factors for Organophosphate Pesticide (OP) Exposure among Indonesian and South Australian Migrant Farmworkers and the Impact of an Intervention to Reduce Exposure

You are invited to participate in a research project examining pesticide exposure among farmworkers in Indonesia and Australia. Your participation in this study is entirely voluntary and you are free to decline to participate or to withdraw from participation at any time. You may refuse to answer any questions that you feel too personal or intrusive. You have been chosen for the study because you are a farmworker over the age of 18 years. This form is part of a process called ‘informed consent’ to allow you to understand this study before deciding whether to take part.

The project includes observation of your work practices and a questionnaire including details of any health symptoms you may have experienced recently. The project includes a pesticide safety education session and the collection of blood on two occasions.

Aims of the project

The aims of this project are to examine factors (personal characteristics, activities associated with pesticide application, safety knowledge, application method, application time, self-protection methods used, and type of packaging of pesticide products) that may contribute to high pesticide exposure. This study will take approximately 6 months to complete with us visiting you on just 2 occasions.

Summary of procedures

- This study consists of two visits to your workplace. The first visit aims to carry out initial baseline measurements and the second to see if these have changed over time.
- At both visits, participants will complete a questionnaire (taking about 20 minutes) about their personal characteristics and how normal work procedures are performed. In addition, we will ask you to provide a very small blood sample from a fingerprick using a single-use sterile needle. This will be used to measure an enzyme in your blood (cholinesterase) that may be affected by pesticide exposure.
- At the end of the first sampling, we will offer you a short information/training session of about 60 minutes to help you improve your work practices to reduce your exposure to pesticides.

The benefits of this study to you are to use the information session and the finger prick blood sample data to determine how much exposure to pesticides you experience during work. You will also be offered information to reduce your exposure and this may result in improved health.

Participation in this study should cause no significant risks or discomfort to participants. If you suffer injury as a result of participation in this research or study, compensation might be paid without litigation. However, such compensation is not automatic and you may have to take legal action to determine whether you should be paid.

Under Australian privacy law all information we have collected about you must be kept confidential, unless you agree to it being released. If you consent to take part in this study no information that could identify you will be given to anyone else, except if required by law. The project outcomes will be published in conference papers, journals or other venues but you will not be identified by name in any report or publication. Records and data about your participation in this study may be used for study purposes, or for further analyses in the future. All such records and your right to them will be protected in accordance with Australian law. No tissue samples (blood) will be retained at the conclusion of this project.

You will not receive any payment for participation in this study.

You may ask any questions you have now. Or if you have questions later, you may contact the researchers via 08 72218560 or sura0018@flinders.edu.au. If you want to talk privately about your rights as a participant, you can call Associate Professor John Edwards. He is the researcher's Supervisor from Flinders University who can discuss this with you. His phone number is 08 72218582. This study has been reviewed by the Southern Adelaide Clinical Human Research Ethics Committee (Approval Number 319.13) and Commission on Health Research Ethics, Faculty of Public Health, Diponegoro University, Semarang, Indonesia with approval number: 183/EC/FKM/2013. If you wish to discuss the study with someone not directly involved, in particular in relation to policies, your rights as a participant, or should you wish to make a confidential complaint, you may contact the Executive Officer on 8204 6453 or email research.ethics@health.sa.gov.au.

Respondent number: ☐☐ - ☐☐ - ☐☐
Year Site Subject

Appendix 4

RESEARCH QUESTIONNAIRE

SCHOOL OF THE ENVIRONMENT, FACULTY OF SCIENCE AND ENGINEERING,
FLINDERS UNIVERSITY, AUSTRALIA

Risk Factors for Organophosphate Pesticide (OP) Exposure among Indonesian and South Australian Migrant Farmworkers and the Impact of an Intervention to Reduce Exposure

Group : a. Indonesian Farmworkers ☐
b. South Australian Migrant Farmworkers ☐

Respondent number : ……………………..
Name of respondent : ………………………………………….
Date of Interview : ☐☐ / ☐☐ / ☐☐☐☐
Date Month Year

Respondent number: ☐☐ - ☐☐ - ☐☐
Year Site Subject

SECTION I

1. PERSONAL CHARACTERISTICS

First, I would like to ask some questions about your personal characteristics.

Q #	Questions	Categories	Answer
1	What is your age?		☐☐ years
2	In what month and year were you born?	Month …………….. Year ………………. Don't know	☐☐ ☐☐ ☐☐
3	What is the highest level of formal education that you have completed?	1. None 2. Elementary school 3. Junior high 4. Senior high 5. Diploma (D1/D2/D3) 6. University	☐
4	How long have you been working as a farmworker?		☐☐☐ months

2. PESTICIDE SAFETY KNOWLEDGE

A. KNOWLEDGE ABOUT ADVERSE EFFECTS OF OPs

Q #	Statements	T	F	DK
A1	OP is not one of the insecticide types	☐	☐	☐
A2	Fungicides are more toxic than insecticides	☐	☐	☐
A3	Insecticides are not harmful for human health	☐	☐	☐
A4	Farmworkers can suffer from pesticide poisoning when they are applying OPs on crops	☐	☐	☐
A5	OPs can enter the body through inhalation	☐	☐	☐
A6	Headache, nausea, cough, and sore throat after applying OPs on crops are not symptoms of pesticide poisonings	☐	☐	☐
A7	Vomiting, sweating, chest pain, and diarrhoea are the symptoms of mild pesticide poisoning	☐	☐	☐
A8	Pesticide poisonings can occur even when farmworkers wash their hands before eating and drinking	☐	☐	☐
A9	OPs will not cause death unless it is swallowed	☐	☐	☐
A10	Psychic disturbances or hallucinations are not symptoms of pesticide poisonings	☐	☐	☐
A11	Risk of pesticide poisoning can be reduced by washing hands using clean water and soap before eating and drinking	☐	☐	☐
A12	OP insecticides are the most toxic pesticides	☐	☐	☐

Abbreviation: T, true; F, false; DK, don't know; OPs, organophosphate pesticides; OP, organophosphate

Respondent number: ☐☐ - ☐☐ - ☐☐

Year Site Subject

B. KNOWLEDGE ABOUT SELF-PROTECTION FROM OP EXPOSURE

Q #	Statements	T	F	DK
B1	Clothing contaminated by OPs is not a factor contributing to pesticide poisonings	☐	☐	☐
B2	Smoking in the field raises the possibility of OPs entering the body	☐	☐	☐
B3	Throwing away empty pesticide containers in a farm area is okay because it will not contaminate the environment	☐	☐	☐
B4	Unused OPs must be stored in a ventilated room and separated from pantry or kitchen	☐	☐	☐
B5	Re-entry into a farm area immediately after pesticide spraying without wearing PPE will increase amount of chemical materials absorbed by a human body	☐	☐	☐
B6	Mixing OPs using bare hands is not harmful and will not cause adverse effects on human health	☐	☐	☐
B7	Mostly farmworkers will not suffer from pesticide poisonings even though they do not wear PPE when working	☐	☐	☐
B8	Wearing unwashed clothing after working in a farm area can be related to signs and symptoms of pesticide poisonings	☐	☐	☐
B9	Pesticide poisonings may occur even if farmworkers shower immediately after working	☐	☐	☐
B10	Wearing PPE is one of the ways to reduce and to prevent pesticide exposure during and after working in farm area	☐	☐	☐

Abbreviation: T, true; F, false; DK, don't know; OPs, organophosphate pesticides; PPE, personal protective equipment; OP, organophosphate

3. PERCEPTIONS ABOUT OP EXPOSURE

PERCEIVED SUSCEPTIBILITY

I am going to ask your opinion about to what extent a farmworker has a possibility to have an adverse effect due to OP exposure.

Q #	Statements	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
C1	Exposure to OPs will not cause any adverse effects to me	☐	☐	☐	☐	☐
C2	Other farmworkers may suffer from pesticide poisoning	☐	☐	☐	☐	☐
C3	Human skin is not a route of OPs to enter the body	☐	☐	☐	☐	☐
C4	OPs are not dangerous for the human body	☐	☐	☐	☐	☐
C5	OPs are not harmful to the body as long as they are not swallowed	☐	☐	☐	☐	☐
C6	Following pesticide exposure, the pesticide is removed by the liver	☐	☐	☐	☐	☐

Respondent number: ☐☐ - ☐☐ - ☐☐
Year Site Subject

PERCEIVED SEVERITY

I am going to ask your opinion about the severity of OP exposure suffered by a farmworker.

Q #	Statements	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
C7	If the pesticide is on the skin, it will only cause a mild effect and it will recover soon	☐	☐	☐	☐	☐
C8	OPs only cause itchy skin	☐	☐	☐	☐	☐
C9	The effect of pesticide on the body is easily cured	☐	☐	☐	☐	☐
C10	Redness on the skin after working with OPs in the fields is not harmful because it is only as an effect of sunlight exposure	☐	☐	☐	☐	☐

PERCEIVED BENEFITS

I am going to ask your opinion about to what extent a farmworker will derive some benefit from an activity to prevent and reduce OP exposure.

Q #	Statements	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
C11	Use of PPE will protect the body from adverse effects of pesticide exposure	☐	☐	☐	☐	☐
C12	Although a bit troublesome, wearing PPE is necessary to improve health	☐	☐	☐	☐	☐

PERCEIVED BARRIERS

I am going to ask your opinion about to what extent a farmworker has barriers to prevent and reduce OP exposure.

Q #	Statements	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
C13	Use of PPE is troublesome	☐	☐	☐	☐	☐
C14	PPE is expensive	☐	☐	☐	☐	☐
C15	Use of PPE causes an uncomfortable feeling in the work	☐	☐	☐	☐	☐
C16	Following all pesticide safety procedures is not efficient because it will need extra time to finish my farm work	☐	☐	☐	☐	☐

Respondent number: ☐☐ - ☐☐ - ☐☐
Year Site Subject

CUES TO ACTION

I am going to ask your opinion about to what extent other factors may influence the perception of individual and directly influence health-related behaviours.

Q #	Statements	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
C17	A health worker often reminds me to use PPE when I am working	☐	☐	☐	☐	☐
C18	My friends were ever sick due to not following pesticide safety procedures during work	☐	☐	☐	☐	☐
C19	My body often feels itchy after using OPs without wearing PPE	☐	☐	☐	☐	☐
C20	I often feel dizzy after spraying OPs on crops	☐	☐	☐	☐	☐

Abbreviation: OPs, organophosphate pesticides; PPE, personal protective equipment

4. HEALTH STATUS

During the past 4 weeks, have you ever experienced **symptoms** below? How many times have you experienced these **symptoms**?

Please tick the appropriate box ☑

- ☐ Weakness _____ times
- ☐ Headache _____ times
- ☐ Dizziness _____ times
- ☐ Nausea _____ times
- ☐ Vomiting _____ times
- ☐ Diarrhoea _____ times
- ☐ Chest pain _____ times
- ☐ Blue lips _____ times
- ☐ Heart palpitations _____ times
- ☐ Dry mouth _____ times
- ☐ Salivation _____ times
- ☐ Watery eyes/teary eyes _____ times
- ☐ Sweating _____ times
- ☐ Difficulty working _____ times
- ☐ Psychic disturbances/ Hallucinations _____ times
- ☐ Fasciculation (twitching) _____ times
- ☐ Hospitalization _____ times
- ☐ Other (please specify) _____ times
  ____

Respondent number: ☐☐ - ☐☐ - ☐☐
Year Site Subject

SECTION II

5. ACTIVITIES ASSOCIATED WITH OP PESTICIDE APPLICATION

Now, let us talk about activities associated with pesticide application.

1) What crops have you worked during the last 3 months?

Please tick the appropriate box ☑

- ☐ Fruits (e.g. Mangoes, Melons, Pears, Stone fruits, etc.)
- ☐ Salad vegetables (e.g. Leeks, Onions, Shallots, etc.)
- ☐ Other vegetables (e.g. Cabbages, Pumpkins, etc.)
- ☐ Genetically modified foods (e.g. Soybeans, Corns, Canola, Potatoes, Tomatoes, etc.)

Q #	Statements	Yes/No
2)	I personally mixed OP pesticides for farm purposes in the last three months	☐
3)	I personally loaded OP pesticides for farm purposes in the last three months	☐
4)	I personally sprayed my crops in the last three months	☐
5)	I touched crops or plants after OP pesticides had been applied in the last three months	☐
6)	I rode equipment, such as a tractor or harvester for farm purposes in the last three months	☐

6. OP PESTICIDE APPLICATION METHOD

Now, let us talk about pesticide application method.

1) What methods do you usually use for applying OP pesticides to crops?

Please tick the appropriate box ☑

- ☐ Do not apply to crops
- ☐ Distribute granules
- ☐ Inject OP pesticides into plant
- ☐ Seed treatment
- ☐ Gas canister
- ☐ Aerial (aircraft application)
- ☐ Backpack sprayer
- ☐ Hand spray gun
- ☐ Airblast
- ☐ Mist blower/fogger
- ☐ Other ____________________________

2) What types of OP pesticides do you usually use on crops?

- ☐ Insecticides
- ☐ Herbicides
- ☐ Fungicides
- ☐ Rodenticides
- ☐ Other (please specify) ______________________

Respondent number: ☐☐ - ☐☐ - ☐☐
Year Site Subject

3) How do you usually pour the chemicals into the application tank when mixing OP pesticides?

☐ Pour into tank by hand ☐ Other (please specify) ________________

4) What kind of equipment do you usually use to stir the mixture when mixing OP pesticides?

☐ Hand/Arm ☐ Automatic stir
☐ Stick/Paddle ☐ Other ________________

5) What additives do you use when mixing OP pesticides?

☐ Water ☐ Solvents
☐ Fertilizer ☐ Other (please specify)
☐ Surfactants ________________

6) Do you use a towing vehicle, such as tractor, trailer, or truck when applying OP pesticides?

☐ Yes ☐ No ==> **If the answer is NO, please jump to number 8**

7) **IF No. 6 YES**, is a vehicle regularly washed?

☐ Yes: ☐ No

a. Daily ☐
b. Weekly ☐
c. Monthly ☐
d. Other (please specify) ☐
____

8) Do you spray OP pesticides on crops?

☐ Yes ☐ No ==> **If the answer is NO, please jump to number 10**

9) How do you spray OP pesticides on crops?

☐ Wind direction ☐ Against wind direction

10) When you apply OP pesticides, do you avoid spray drift?

☐ Yes ☐ No

11) When you apply OP pesticides, do you ensure you do not affect other people by over applied spray drift?

☐ Yes ☐ No

Respondent number: ☐☐ - ☐☐ - ☐☐
Year Site Subject

7. OP PESTICIDE APPLICATION TIME

1) When did you last apply pesticides?

- ☐ 1 – 6 days ago
- ☐ 1 – 2 weeks ago
- ☐ 3 – 4 weeks ago
- ☐ 1 month ago
- ☐ Other (specify) ______________________

2) When did you last apply OP pesticides?

- ☐ 1 – 6 days ago
- ☐ 1 – 2 weeks ago
- ☐ 3 – 4 weeks ago
- ☐ 1 month ago
- ☐ Other (specify) ______________________

3) How many times do you spray pesticides on crops per week?

☐☐☐ times.

4) How many times do you spray OP pesticides on crops per week?

☐☐☐ times.

8. SELF-PROTECTION METHODS USED

Please tick the appropriate box ☑

1) Do you protect yourself from OP pesticides when you are working?

- ☐ Yes, Always
- ☐ Yes, Usually
- ☐ Yes, Sometimes
- ☐ No, Never

2) Have you ever received any information or training on how to prevent or reduce pesticide exposure when you are working?

☐ Yes ☐ No ===> **If the answer is NO, please jump to number 4**

3) **IF No. 2 YES**, How many times have you received information or training since you worked as a farmworker until now? ☐☐☐ times.

4) What types of personal protective equipment do you usually wear when you are working?

Please tick the appropriate box ☑

Clothes:

- ☐ Long sleeved shirt
- ☐ Short sleeved shirt
- ☐ Coveralls
- ☐ Long pants/Leg covering
- ☐ Shorts

Respondent number: ☐☐ - ☐☐ - ☐☐
Year Site Subject

Headwear:

- ☐ Wide brim hat
- ☐ Cap
- ☐ No hat

Footwear:

- ☐ Chemically resistant boots or shoes
- ☐ Waterproof boots
- ☐ Sneaker
- ☐ No shoes

Mask:

- ☐ Gas mask, Cartridge mask
- ☐ No mask
- ☐ A filtering facepiece
- ☐ Other mask/respirators (please specify) ______________________

Gloves:

- ☐ Leather gloves
- ☐ Waterproof elbow length gloves
  If yes, what type? ______________
- ☐ Waterproof gloves
  If yes, what type? ______________
- ☐ Other type of gloves (please specify) ______________________________
- ☐ No gloves

Eye Protections:

- ☐ Safety glasses
- ☐ A face shield
- ☐ Chemical goggles
- ☐ Other type of eye protection (please specify) ____________________
- ☐ No eye protection

5) How often do you wash your hands after work using clean water and soap before eating?

- ☐ Always
- ☐ Usually
- ☐ Sometimes
- ☐ Never

6) How often do you wash your hands after work using clean water and soap before touching regular clothes?

- ☐ Always
- ☐ Usually
- ☐ Sometimes
- ☐ Never

7) How often do you take a shower immediately after work?

- ☐ Always
- ☐ Usually
- ☐ Sometimes
- ☐ Never

8) How often do you wear the same clothes more than one day without washing them?

- ☐ Always
- ☐ Usually
- ☐ Sometimes
- ☐ Never

Respondent number: ☐☐ - ☐☐ - ☐☐
Year Site Subject

9. TYPE OF PACKAGING OF OP PESTICIDE PRODUCTS

1) What types of OP pesticides packaging did you use in the last three months?

- ☐ Bags
- ☐ Cans
- ☐ Liquid containers
- ☐ Bottles
- ☐ Other (Please specify) ____________________

2) What brand names of pesticide products did you use (mixing, loading, spraying) on crops in the last three months?

Brand name:

a. ______________________ Code __________ __________

b. ______________________ Code __________ __________

c. ______________________ Code __________ __________

d. ______________________ Code __________ __________

e. ______________________ Code __________ __________

f. Did not mix or apply to crops

10. WORKPLACE CONDITIONS

Now, I am going to ask you some questions about facilities provided in your current work place.

Q #	Statements	Yes/No
1)	There is water for you to drink in the fields	☐
2)	There are enough cups provided to drink using a clean cup for each worker	☐
3)	There is water to wash your hands	☐
4)	Soap is available for handwashing	☐
5)	Single use towels are available for handwashing	☐
6)	Washing water is separated from drinking water	☐
7)	There is any break room to take a rest for meals	☐
8)	There is a toilet facility	☐

11. COMMENTS

……………………………………………………………………………………

……………………………………………………………………………………

……………………………………………………………………………………

……………………………………………………………………………………

Thank you very much for your time in completing this questionnaire.

Appendix 5

DATA SHEET OF AChE AND PChE ACTIVITY LEVELS IN WHOLE BLOOD

SCHOOL OF THE ENVIRONMENT, FACULTY OF SCIENCE AND ENGINEERING,
FLINDERS UNIVERSITY, AUSTRALIA

Risk Factors for Organophosphate Pesticide (OP) Exposure among Indonesian and South Australian Migrant Farmworkers and the Impact of an Intervention to Reduce Exposure

Group : a. Indonesian Farmworkers ☐
b. South Australian Migrant Farmworkers ☐

Date of Measurement : ☐☐ / ☐☐ / ☐☐☐☐
Date Month Year

Erythrocyte Acetylcholinesterase (AChE) and Plasma Cholinesterase (PChE) levels in whole blood:

Resp. Number	Name	AChE (U/mL)	AChE (%N)	Hgb (g/dL)	Hgb (%N)	Q (U/g)	Q (%N)	PChE (U/mL)	PChE (%N)	Hgb (g/dL)	Hgb (%N)

Appendix 6

DATA SHEET OF PChE LEVELS IN FRESH PLASMA BLOOD SAMPLES WITH/WITHOUT PRALIDOXIME

SCHOOL OF THE ENVIRONMENT, FACULTY OF SCIENCE AND ENGINEERING, FLINDERS UNIVERSITY, AUSTRALIA

Risk Factors for Organophosphate Pesticide (OP) Exposure among Indonesian and South Australian Migrant Farmworkers and the Impact of an Intervention to Reduce Exposure

Group : a. Indonesian Farmworkers ☐

b. South Australian Migrant Farmworkers ☐

Date of Measurement : ☐☐ / ☐☐ / ☐☐☐☐

Date Month Year

Resp. Number	Name	PChE - Oxime				PChE + Oxime				Difference [(b – a)/b]*100 (% INH)
		a. PChE (U/mL)	PChE (%N)	Hgb (g/dL)	Hgb (%N)	b. PChE (U/mL)	PChE (%N)	Hgb (g/dL)	Hgb (%N)	

Note: %INH = Percent Inhibition

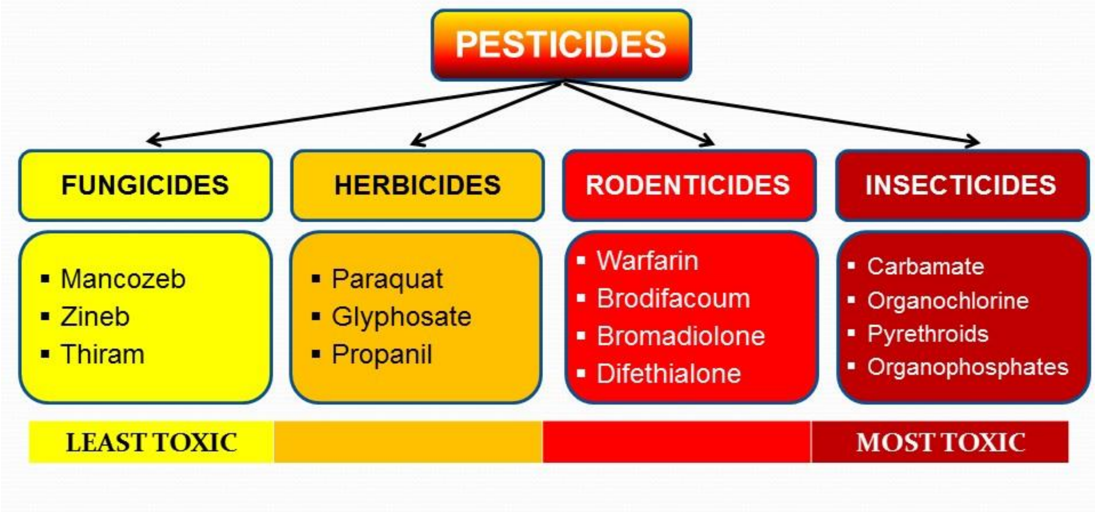
Appendix 7

EDUCATIONAL INTERVENTION MATERIALS

RISK FACTORS FOR ORGANOPHOSPHATE PESTICIDE (OP) EXPOSURE AMONG FARMWORKERS

SURATMAN

Pesticides are natural or synthetic chemicals, mixture of these, or living organism used to prevent, destroy, control, repel, or mitigate any pest, vectors of human or animal disease, unwanted species of plants or animal causing harm either before or after harvest.



EPA (2005); FAO (2009); FIFRA (2008); http://extension.psu.edu/ipm/schools/educators/curriculum/pesticides/chemicalcontrol

GROUPS OF PESTICIDES

- There are any four groups of pesticides:

  Fungicides, Herbicides, Rodenticides, and Insecticides

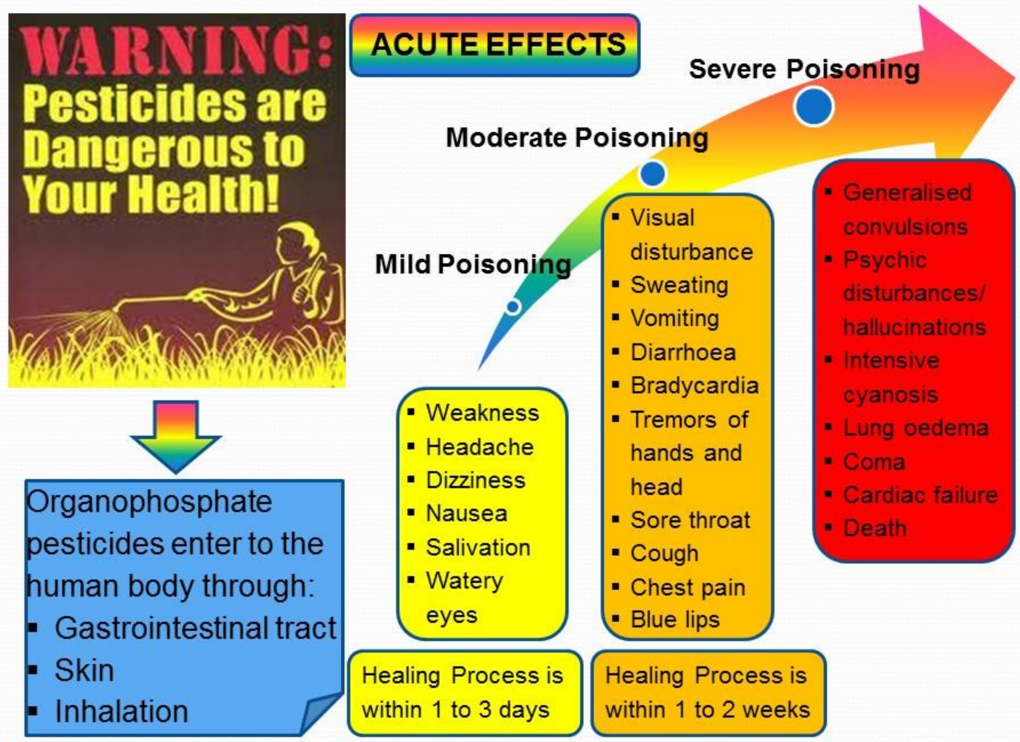
- These four types of pesticides are toxic to humans and can cause poisoning.
- From most to least toxic pesticides consecutively are:
  Insecticides – Rodenticides – Herbicides – Fungicides
- Organophosphate pesticides are the most widely used in the world. It is one of the insecticide types.
- Especially for the insecticides, Organophosphate is the most toxic insecticides.

ORGANOPHOSPHATE PESTICIDE (OP) EXPOSURE

- Farmworkers and their families are at risk to expose to organophosphate pesticides either in a farm area or in their homes.
- Farmer groups can suffer from organophosphate pesticide poisoning when they are applying pesticides on crops.

SOME ACTIVITIES ASSOCIATED WITH THE INCREASE OF ORGANOPHOSPHATE PESTICIDE (OP) EXPOSURE

- Mixing, loading, and spraying pesticides without wearing Personal Protective Equipment (i.e. gloves, mask, long-sleeved shirt, etc.)
- Leaking equipment and mixing with bare hands.
- Clothing contaminated by pesticides.
- Wearing unwashed clothing after working in a farm area.
- Smoking in the fields.
- Throwing away empty pesticide containers in a farm area will raise the possibility of pesticides entering the body and will contaminate the environment.
- These behaviours can be related to sign and symptoms of pesticide poisonings. In addition, pesticide poisonings can occur even when farmworkers wash their hands before eating and drinking



Workplace Health and Safety Queensland (2012), www.worksafe.qld.gov.au

ADVERSE HEALTH EFFECTS OF ORGANOPHOSPHATE PESTICIDE (OP) EXPOSURE

- Exposure to organophosphate pesticides cause adverse health effects and lead to death.
- Toxic effects due to organophosphate pesticide exposure consist of acute and chronic effects.
- Acute effects occur shortly after exposure to pesticides.
- Organophosphate pesticides enter the human body through inhalation, gastrointestinal tract (intentionally or unintentionally swallowing) and skin.
- Acute effects of organophosphate pesticide poisoning consist of three categories, namely mild, moderate, and severe poisoning.

ADVERSE HEALTH EFFECTS OF OP EXPOSURE (CONTINUED…….)

- The symptoms of organophosphate pesticide poisoning are similar to other health problems. Therefore, the link to pesticides may go undetected.
- Headache, nausea, cough, sore throat are some symptoms of pesticide poisoning suffering from farmworkers after applying pesticides.
- In addition, some symptoms of moderate poisoning are vomiting, sweating, chest pain, and diarrhoea. Furthermore, psychic disturbances or hallucinations are part of severe poisoning symptoms.

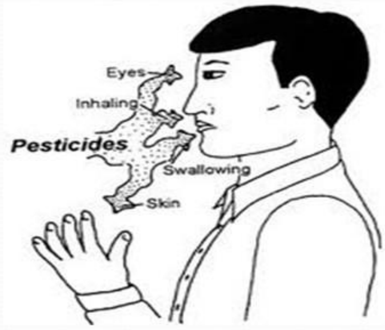
SIGNS OF ORGANOPHOSPHATE PESTICIDE POISONING ON THE PARTS OF THE BODY :

- **Nose & Mouth:** runny nose, drooling
- **Chest & Lungs:** pain, breathing problem
- **Stomach:** pain, diarrhoea, nausea, and vomiting,
- **Legs & Arms:** muscle cramps or pains, twitching
- **Skin:** itching, rashes, bumps, redness, blisters, burning, sweating too much
- **Head & Eyes:** headaches, vision problems, small pupils in the eyes, tears
- **Hands:** damage to fingernails, rashes, numbness and tingling in fingers
- **Other general signs:** confusion, weakness, trouble walking, trouble concentrating, muscle twitching, restlessness and anxiety, bad dreams and trouble sleeping

Jeff Conant , retrieved from: www.hesperian.org

CHRONIC EFFECTS

'Chronic effects are any harmful effects that occur from repeated exposures to small doses of pesticides over an extended period of time.'



'Some chronic effects from exposure to certain pesticides include birth defects; cancers; blood disorders; neurological problems; and reproductive effects (sterility).'

http://doh.state.fl.us/environment/medicine/pesticide/Pesticides_and_Chronic_Effects.html

SELF-PROTECTION FROM ORGANOPHOSPHATE PESTICIDE (OP) EXPOSURE IN A FARM AREA

- Self-protection to pesticide exposure can be done in a farm area and at home.
- It aims to reduce and to prevent pesticide exposure during and after working in the fields.
- Protective clothing, such as long-sleeved shirt, knee-high or long pants, and coveralls, and Personal Protective Equipment, such as chemical-resistant gloves, eyeglasses, headwear, and footwear, are often needed during pesticide handling and application.
- They can reduce dermal contact and inhalation exposures.

SELF-PROTECTION FROM OP EXPOSURE IN A FARM AREA (CONTINUED……)

- Not smoking in a farm area;
- Not re-entry into a farm area immediately after pesticide spraying;
- Always washing hands using clean water and soap before eating and drinking when a break time;
- Always reading the label before using any pesticide is one of the ways to prevent inappropriate pesticide handling;
- Unused pesticides must be stored in a ventilated room and separated from pantry or kitchen.

SELF-PROTECTION FROM ORGANOPHOSPHATE PESTICIDE (OP) EXPOSURE AT HOME

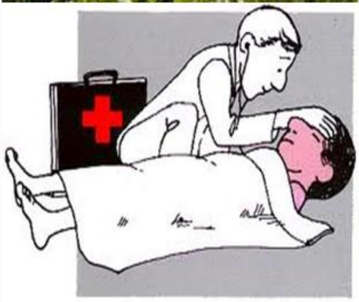
- Wash your hands with clean water and soap before eating, drinking, touching your eyes, nose, or mouth;
- Wash after working with pesticides;
- Wash your clothes with care after working with pesticides apart from regular or family clothes;
- After work, change into clean clothing; 4) wash fruits, vegetables, and other foods well in salt water (5 spoonfull of salt to 1 liter of water) to reduce the amount of pesticide residue, then rinse in fresh water;

SELF-PROTECTION FROM OP EXPOSURE AT HOME (CONTINUED…….)

- Do not eat the outer leaves of leafy greens like cabbage and lettuce, because these are the parts that collect the most pesticides;
- Shower immediately using clean water and bath soap after working in a farm area;
- Do not put unused pesticides in pantry or kitchen and keep pesticides away from children;
- Get rid of empty pesticide containers, the best thing to do with empty pesticide containers is to bury them.

PERSONAL PROTECTIVE EQUIPMENT

WHAT DO I DO IF I AM EXPOSED?



- If you are exposed to pesticides through skin (mixing, spraying, or touching crops that just have been sprayed), remove clothing the pesticides spilled on, and wash yourself with soap and cool water soon and change into clean clothing.
- If you are exposed to pesticides through mouth, drink lots of clean water
- If you are exposed to pesticides through inhalation, get away from the pesticides soon! Do not wait until you feel worse
- If you are ill or injured, visit your health care provider

Jeff Conant , retrieved from: www.hesperian.org