CHAPTER 5

DISCUSSION

5.1 Pesticide usage

In this study, herbicides account for the highest proportion of total pesticide usage, followed by fungicides and insecticides. The greater proportion of herbicides resulted from preparation for cultivating strawberry crops. Strawberries perform best on well-drained soils and good air drainage to avoid and decrease risk of black root rot and leaf diseases. Weed control is important in field, shelterbelts, fence lines and areas surrounding because weeds can keep relative humidity high and slow drying. Thereby the high humidity encourages strawberry risk from plant diseases, especially fungal diseases.

Early in the growing season, soil was broken up and weeds removed by spraying herbicides, whereas fungicides and insecticides were applied during strawberry cultivation.

In growing season, ammonium and organophosphorus herbicides were highly used in strawberry cultivation, because both of them are broad spectrum, nonselective systemic herbicides, used for annual and perennial plants. They also can be used on non-cropland as well as on a great variety of crops.

Fungicides and insecticides were also used in the growing season. Conazole fungicides were used during strawberry transplantation and growing to protect strawberry against fungal diseases. Ethylene bis dithiocarbamate, a carbamate insecticide, was used to protect many fruit and strawberry for pests control.

Amounts of pesticides used in harvesting season were decreased, because most strawberries are harvested in the winter season when pests and insects that attack and destroy strawberries are low. However, herbicides were still being used in a high proportion of pesticides in harvesting season.

5.2 Pesticide exposure and Health impact

Most of farmers in this study used personal protection equipments and had awareness of pesticide mixing and spraying from the right direction of wind. Only a small farmer group was mixing pesticide with bare hands, kept pesticide in areas close to children or smoked during spraying.

In this study, dermal and respiratory exposures were more likely occurring. Hands, eyes and inhalation route were likely heavily contaminated as few use rubber gloves, glasses and masks. Most farmers reported using gloves but they always got hands soaked with pesticide after spray operation, because their gloves were not rubber gloves. The other routes of pesticide exposure in this group were taking food or water during spraying without washing or changing their clothes and getting wet during spraying pesticides. Approximately 48% and 45% believed that their faces and hands were the major part of body exposed to pesticides.

The New York State Pesticide Poisoning in 1998 reported that the major route of pesticide exposure was inhalation and approximately half involved dermal exposure (Pataki G.E. 1998). In Cambodia, Approximately 28% of the farmers specified that pesticides enter their body through the skin and inhalation route (Sodavy and et al. 2000). The observation data of pesticide exposure in Indonesian farmers showed relationship between the appearance of signs and symptoms and skin exposure (Kishi M and et al. 1995). Dermal exposure is the most important route of entry and an important risk factor for pesticide poisoning.

The signs and symptoms of pesticide poisoning found frequently in farmers were dizziness (50%), fatigue (39%), dry or sore throat (37%), excessive sweating (29%), muscle weakness (23%) and others. The symptoms in humans, which generally occur when acetylcholinesterase activity has been reduced by about 50%, may include: headache, exhaustion and mental confusion together with blurred vision, sweating, salivation, chest tightness, muscle twitching and abdominal cramps. The more severe effects can include muscle paralysis leading to severe difficulty in breathing, so requiring respiratory support (PSD 2003).

The observation of signs and symptoms through self report could be used as a preliminary tool to monitor pesticide poisoning in farmers and occupational pesticide exposures. The most common signs and symptoms were headache, dizziness, excessive sweating and salivation (Pataki G.E. 1998, Sodavy and et al. 2000, Kishi M and et al. 1995, Nordini and et al. 2002). However, the observation of signs and symptoms may also include the effect of hard work and hot weather.

5.3 Cholinesterase enzyme activity and its genetic variants

In this study, mean AChE activity in growing season was significantly lower than AChE activity in harvesting season ($p<0.001$, paired t-test). BuChE activity between growing was significant higher than BuChE activity in harvesting season ($p<0.001$, paried t-test).

There are 4 reasons why the low activity of AChE found in growing season was raised in harvesting season. First, Acetylcholinesterase enzyme is commonly found in red blood cells and its characteristic slowly recovers and regains the higher enzyme activity after pesticide inhibition. Second, blood collection must be drawn during actual or immediately after exposure to organophosphate and carbamate pesticides. The enzyme levels may return to baseline level within 4 hours of exposure (Exotoxnet, 2004). In fact, pesticide spraying operation is always conducted in the evening, while our study collected blood sample in the next day morning. Third, recovery depends on elimination of the OP product from the body and return of acetylcholinesterase activity (PSD, 2003). Fourth, the other studies recommended that

AChE in red blood cells is not a good indicator of pesticide exposure, because its activity is less sensitive than BuChE in plasma (TGA, 2000; Rosmann, 2003).

The investigation of BuchE found that BuchE activity in growing season was significantly higher than BuchE activity in harvesting season ($p<0.001$, paired t-test). Herbicides were heavily used early in the growing season for soil preparation. Otherwise, insecticides and fungicides were applied more in fields after crops were planted. The low level of BuchE activity might be results of low dose of pesticide exposure and directly exposed through their dermal route during harvesting their crops. AchE activity was not decreased more, because AchE inhibition was related to acute pesticide poisoning, and BuchE activity can be expected to return to normal at the rate of approximately 1% per day, providing no further absorption of the pesticide occurs.

Medical or physician guidelines usually recommended observing the baseline cholinesterase level on each occupational pesticide worker. Variation from the baseline enzyme level might be reasonably due to pesticide exposure.

In fact, farmers' with especially small holding in Thailand carried on cultivation and field work all year round. Cholinesterase activities did not result from pesticide using in only a particular growing or harvesting seasons. Measurements indicate the course of the recovery.

Another factor that should be under investigation following the cholinesterase enzyme level is the genetic variants of cholinesterase. We found four phenotypes in this population including UU, UA, UF and AF. The highest activity was found in UU and UF phenotype, while the lowest activity and intermediate level were found in AF and UA, respectively. Farmers with UU and UF phenotype had the highest level of ChE. There were also found more farmers reported more signs and symptoms more than the farmers who have UF and AF phenotype. The only measurement of BuchE and AchE alone might not be enough to point out the risky group, because of the low level of enzyme might be the result of their genetic variants (Kalow 1991, Goodall 2004).

5.4 Paraoxonase activity and its genetic polymorphism

Paraoxonase enzyme has been proposed as pesticide intoxication indicator through its hydrolysis of paraoxon, which is an active form of organophosphate or carbamate pesticide produced from microsomal cells. The reaction of paraoxon hydrolysis results in a decrease in toxicity. Its metabolites (dialkylphosphate metabolites) do not inhibit cholinesterase enzymes (Furlong 1988).

In this study, Paraoxonase enzyme, which is responsible for hydrolysis and deactivation of organophosphate is under investigation as a biomarker of pesticide susceptibility There is no significant difference between the paraoxonase activity between growing and harvesting season at p>0.05(paired t-test), results of this enzyme is concerned as protective or susceptibility to organophosphate than toxicity effects. Paraoxonase enzyme is one important enzyme that is under investigation as a biomarker of pesticide susceptibility, which is responsible for the hydrolysis and deactivation of organophosphate (Furlong 1991, Mackness 1997b, Costa 1999 and Akgur 2003).

In this study, Pearson's value showed the correlation between paraoxonase and AchE activity at $p<0.05$. Organophosphate and carbamate pesticides share a common mechanism of toxicity through inhibitory effects on cholinesterase enzymes in the nervous system. Organophosphates contain central phosphorus with a double bond to either sulfur or oxygen. The chemical reaction occuring in humans is a transformation at the double bond of central phosphorus atom from sulfur to oxygen, resulting in activation of organophosphate to a more potent inhibitor of cholinesterase enzymes.

Another metabolic reaction that occurs in human body is hydrolysis of organophosphate by paraoxonase enzyme, yielding a dialkylphosphate and the leaving group. This reaction results in a decrease in toxicity, as the leaving group and dialkylphosphate metabolites do not inhibit cholinesterase enzymes. The positive correlation between paraoxonase and cholinesterase enzyme might resulted because detoxification reaction occurs more than activated organophosphate to be a cholinesterase inhibitor (La Du, 1999; Sogorb, 2002; Costa, 2003).

The polymorphisms in the PON1 gene that exist in humans may affect the efficiency with which an individual will metabolize organophosphates. Biomarkers of genetic susceptibility may prove to be important in assessing an individual's risk of adverse effects from excessive exposure. Many reports showed the correlation between paraoxonase level and PON1 gene loci 192 more than the correlation to PON1 loci 55 (Cheery, 2002; Li, 2000; Costa, 1999 and Allebrandt, 2002).

The frequency of allele R and Q on PON1 gene loci 192 were 0.54 and 0.46, respectively. On PON1 gene loci 55, the frequency of allele M and L were 0.02 and 0.98, respectively. Pearson's value show the positive correlation between loci 192 at $p<0.05$ (Peason's value 0.736, $p<0.05$, ANOVA), while the negative correlation showed in the PON1 loci 55 (Pearson's value -0.101, $p<0.01$, unpaired t-test). The frequency of R allele on PON1 loci 192 and frequency of M allele on PON1 loci 55 were compared to other studies and shown that R allele is frequently found in oriental population. A very low frequency of M allele was found in this study, the same as other reports which studied oriental population. (Allebrandt, 2002; Sanghera, 1998; Suehiro, 1997 and Imai, 2000).

However, Paraoxonase enzyme is a new biomarker of pesticide poisoning. There was no normal range for PON in each population to identify the risk group to pesticide intoxication. The measurement of PON level might be used as a tool to categorize the risk group enough for monitoring in the occupational pesticide exposure mostly in the farm workers.