CHAPTER 6

CONCLUSION

6.1 Conclusion of this study

- A large proportion of pesticide usage in growing season was herbicides, insecticides and fungicides, respectively. The greater proportion of herbicides applied in growing season was for soil preparation. Amount of pesticide use was reduced in harvesting season when the greater proportion of herbicides was still applied in this season for weed control to avoid and decrease risk of black root rot and leaf diseases. Insecticides and fungicides were also applied in field for crops protection against pests, insects and fungal diseases.

- Data of signs and symptoms of pesticide poisoning were obtained on individual farmers. Some signs and symptoms might be the result of the effect of hard working and hot weather. Our interviewers always asked farmers to give their opinions and specify that all signs and symptoms were the result of pesticide exposure. The frequent signs and symptoms were fatigue, dizziness, red eyes and muscle weakness, which were mostly found in growing rather than harvesting season.

- Farmers' AchE in growing season was significantly higher than AChE level in harvesting, although some studied recommended that AChE is a good indicator for acute poisoning and more accurate than BuchE. AchE also could be used as a measure of pesticide long term exposure due to it's slow recovery of enzyme activity. The higher level of AchE in harvesting season might be the result of the recovery and regain of the individual baseline level.

- Farmers' BuchE in growing season was significantly lower than BuchE level in harvesting season. BuchE is more labile and recovers more quickly. BuchE is suitable for assessment of current or recent exposure to pesticide.

- Cholinesterase genetic variants were observed in this population, and 3 phenotypes, UU, UA and AF, were found. The highest BuchE activity was found in UU phenotype. UA phenotype showed intermediate enzyme activity and AF showed the lowest enzyme activity. The consideration of phenotype and BuchE activity should be done together, because the low level of enzyme activity might be the result of individual genetic variants, not result of pesticide poisoning. Other factors which effect cholinesterase level are sex, age, body weight, height and health status such as pregnancy or malnutrition, and some diseases such as liver disease, Parkinson's disease, and Alzheimer's disease.

- There is no significant difference between paraoxonase enzyme level in growing and harvesting season, because this enzyme is concerned as protective or susceptibility to organophosphate rather than toxicity effects. In these two last decades it has been demonstrated that PON's ability to protect against pesticides toxicity is by hydrolyzing organophosphate pesticides. The efficiency of hydrolyzing afforded a degree of in vivo protection by each isoform. Low PON1 activity may also contribute to the higher sensitivity to organophosphate toxicity.

- There is a positive correlation between PON and ChE level. The more PON activity will more detoxify of organophosphate and carbamate pesticide. PON should be used as pesticide biomarker of susceptibility, although the normal range of PON is still not established.

- Pearson's values show the positive correlation between loci 192, while the negative correlation showed in the PON1 loci 55. The measurement of PON level might be used as a tool to point out the risk group and enough for monitoring in the occupational pesticide workers. The A isoenzyme of human paraoxonase has a much better catalytic efficiency and may have increased resistance to paraoxon. It is expected that oriental populations show greater resistance to paraoxon and less resistance to neurotoxic agents such as sarin, soman and diazoxon compared to Caucasians.

- Frequency of allele Q and R on PON1 loci 192 and allele L and M on PON1 loci 55 were close to another study in the oriental population. Genetic polymorphism of both PON1 loci 192 and 55 depends on race and ethnicity in each population. The genotype frequency of R allele polymorphism in the Thai population of this study and in Chinese, Taiwan and Japanese are higher than Caucasian groups.

6.2 Limitation of this study

- Blood cholinesterase measurements have limitations in that the time course for enzyme inhibition and recovery can vary with exposure to different organophosphates. Cholinesterase activity can also be affected by inter and intra individual variability. In an asymptomatic, healthy individual without a history of recent overexposure to organophosphate insecticides, cholinesterase testing is not likely to be of clinical value.

- Many factors that decrease cholinesterase activity include hypersensitivity to drug or derivatives, severe liver disease, peptic ulcer disease, asthma, chronic obstructive, pulmonary disease, and current alcoholism. Contraindications to increase enzyme activity were the side effects of medication and dosage used and gastrointestinal disease. All factors were included in the questionnaire, but most of farmers did not recognize names of medicine, some of them took herbal medicine, while the others guessed

and treated their diseases without physician's diagnosis. Subjects who had cholinesterase affectors should be excluded from this study.

- The method determination of cholinesterase phenotype, the standard method, was determination of dibucaine and fluoride number. This method might miss detection of some of the BuChE variants that cause sensitivity to other BuChE substrates such as succinylcholine and benzoylcholine. Other variants difficult to detect by this standard method are K and J variants. However, worldwide centers have made an effort to provide the service and stock all the required PCR amplification primers as well as provide the amplification and sequencing facilities for BuChE genetic determination. But very few laboratories can perform and provide these facilities.

- In this study, because of a small sample size and small research budget, our findings are preliminary. Nevertheless, it calls for further investigation of the interaction between the PON1 genotype and organophosphate pesticide exposure in high organophosphate pesticide exposure groups.

- In this study, we conducted the prospective follow up study in growing and harvesting season. Only 100 subjects participated in this study, while the other follow up studies involved a few hundred or a few dozen individuals. At the time of giving consent, all participants will have been asked for explicit written permission for follow-up and to contact them again in the next season (harvesting season). In the harvesting season, farmers were harvesting their crops and did not have time to participate in the second follow. Farmers change their mind and it was a potential difficulty with follow up.

- An outcome such as signs and symptoms of pesticide poisoning are difficult to identify, because acute poisoning of pesticides may take a high dose of exposure to develop. Many signs and symptoms are affected of other conditions such as hard working, working in the hot weather and taking some medicines.

6.3 Recommendation

- The conduct of this study conformed to the relevant ethical and legal guidelines regarding consent, confidentiality and the use of human blood samples. The project underwent extensive ethical review required for approval by the Research Ethics Committee in Research Institute for Health Sciences, Chiang Mai University. The conduct of studies in human must conform to the ethical committee and follow the medical guidance for human biological sample collection.

- Although, dibucaine and fluoride numbers were used as standard method for determine of BuChE genetic variants, the molecular biology of amplification and sequencing for BuChE genetic determination must be the best method to determine the genetic variants of BuChE.

- The study of cholinesterase enzyme activity must also consider enzyme affectors which result in cholinesterase misinterpretation during conduct of the research project.

6.4 Future research study

- Acetylcholinesterase, Butylrylcholinesterase and paraoxonase enzymes in farmers must be compared with other populations such as consumers and occupational pesticides workers.

- The normal level of paraoxonase enzyme activity and distribution of PON1 genetic polymorphism have not been established in many ethnic groups or in Thai population. This study was a preliminary research project in a small group of Thais. The future study must be conducted in a larger sample size and should set up the normal level of paraoxonase enzyme and understand PON1 genetic polymorphism in Thais.

- Determination of enzyme activity and genetic variants of cholinesterase and paraoxonase enzyme might be used as a tool to identify the risk of individual workers to pesticides exposure. Furthermore, all of these results might be used to establish guidelines for use of pesticides by farmers and occupational pesticides workers.