PESTICIDES EXPOSURE AND HEALTH RISK ASSESSMENT: A CASE STUDY OF HEALTH EFFECT ON MALE GROUND-NUT FARMERS' REPRODUCTIVE SYSTEM IN KYAUK-KAN VILLAGE OF NYAUNG-U DISTRICT, MANDALAY REGION, MYANMAR

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การสัมผัสสารกำจัดศัตรูพืชส่งผลต่อหลายอวัยวะในร่างกายรวมทั้งระบบสืบพันธุ์ งานวิจัยนี้เป็นการศึกษาวิจัยแรกในประเทศสาธารณรัฐแห่งสหภาพเมียนมา โดยมีวัตถุประสงค์ในการศึกษาความรู้และการปฏิบัติตนต่อการใช้สารกำจัดศัตรูพืชและศึกษาผลกระทบต่อระบบระบบสืบพันธุ์ในเพศชาย โดยใช้ตัวบ่งชี้ทางชีวภาพ ได้แก่ น้ำเชื้อ ซีรัมฮอร์โมน และ ระดับเอนไซม์โคลีนเอสเทอเรสในเลือดของเกษตรกรชายในหมู่บ้านคชาวค์ คาน เขตนวงยู มัณฑะเลย์ ประเทศสาธารณรัฐแห่งสหภาพเมียนมา การศึกษานี้แบ่งออกเป็น 3 ช่วงการศึกษา คือ

ช่วงที่ 1 การศึกษาชนิดภาคตัดขวาง โดยการเก็บข้อมูลปัญหาสุขภาพที่เกี่ยวข้องกับการสัมผัสสารกำจัดศัตรูพืช และศึกษาความรู้และการปฏิบัติตนต่อการใช้สารกำจัดศัตรูพืชในเกษตรกรผู้ปลูกถั่วลิสงทั้งชายและหญิง (n=400) ในชุมชนโดยใช้แบบสอบถามและการสัมภาษณ์ หนึ่งในสามโดยประมาณของผู้เข้าร่วมการศึกษา มีอายุระหว่าง 38-47 ปี ประมาณครึ่งหนึ่ง (ร้อยละ 53.5) มีระดับความรู้ปานกลาง และ ร้อยละ 79.2 มีการปฏิบัติตนอยู่ในระดับต่ำ โดยเกษตรกรส่วนใหญ่ทั้งชายและหญิงไม่ปฏิบัติและใช้สารกำจัดศักตรูพืชเกินกว่าคำแนะนำ และพบว่าลักษณะทางเศรษฐสังคมของผู้เข้าร่วมวิจัยไม่มีความสัมพันธ์กับระดับความรู้และการปฏิบัติตนต่อการรับสัมผัสและป้องกันตน

ช่วงที่ 2 การศึกษาชนิดภาคตัดขวาง โดยการเก็บตัวอย่างดัชนีชีวภาพ ผู้เข้าร่วมวิจัยเกษตรผู้ปลูกถั่วเพศชายจำนวน 100 คน โดยวิธีการสุ่ม จากช่วงที่ 1 พบว่าเกษตรผู้ปลูกถั่วเพศชายมีอายุเฉลี่ย (± ส่วนเบี่ยงเบนมาตรฐาน) 37.51 ± 9.45 ปี และ ครึ่งหนึ่งมีระดับการศึกษาชั้นประถม และพบว่าผลกระทบจากการใช้สารเคมีทางการเกษตรอย่างต่อเนื่องต่อระบบสืบพันธุ์ของเกษตรผู้ปลูกถั่วเพศชาย การเปรียบเทียบความแตกต่างของดัชนีชีวภาพในช่วงทำการเกษตรและไม่ทำการเกษตรโดยใช้สถิติทดสอบวิลคอกซัน (The Wilcoxon Signed - Rank Test) พบความแตกต่างอย่างมีนัยสำคัญ ($p<0.05$) ของ ค่าความเป็นกรดด่าง ความหนืด การเคลื่อนไหว สัณฐานวิทยาและจำนวนตัวอสุจิในน้ำเชื้อ การเปลี่ยนแปลงฟอลลิเคิลสติมิวเลติงฮอร์โมน ระดับเอนไซม์โคลีนเอสเทอเรสในเลือด เอมไซม์อะซีติลโคลีนเอสเทอเรสในเม็ดเลือดและในพลาสม่า นอกจากนี้พบความสัมพันธ์อย่างมีนัยสำคัญ ($p<0.05$) ของดัชนีชีวภาพที่เกี่ยวข้องกับการสัมผัสสารกำจัดศัตรูพืชและการทำงานในช่วงทำการเกษตรและไม่ทำการเกษตรโดยใช้สถิติการวิเคราะห์การถดถอยโลจิสติก ผลการศึกษาช่วงที่ 2 ชี้ให้เห็นว่าการสัมผัสสารกำจัดศัตรูพืชในระยะยาวถึงแม้จะมีปริมาณน้อยมีความสัมพันธ์กับระบบสืบพันธุ์และการลดลงของคุณภาพน้ำเชื้อ

ช่วงที่ 3 การประเมินผลกระทบสุขภาพ โดยวิธีการเช็ดผิวหนัง (มือ) ของเกษตรกรผู้ปลูกถั่วลิสงชายจำนวน 30 คน โดยวิธีการสุ่มเกษตรกรจากช่วงการศึกษาที่ 2 พบปริมาณการสัมผัสต่อวัน 3.66×10^{-5} มิลลิกรัมต่อกิโลกรัม-วันในช่วงทำการเกษตร การประเมินความเสี่ยงชนิดไม่ก่อให้เกิดมะเร็งจากการรับสัมผัสสารกำจัดศัตรูพืชคลอไพริฟอส กลุ่มออร์แกนโนฟอสเฟต พบว่าเกษตรกรผู้ปลูกถั่วลิสงชายมีแนวโน้มไม่เสี่ยงต่อการสัมผัสสารดังกล่าวผ่านทางผิวหนัง (มือ) โดยมีค่าดัชนีสารอันตราย (Hazard Quotient, HQ) เท่ากับ 0.12 (ต่ำกว่าเกณฑ์ที่ยอมรับได้ คือ มีค่าน้อยกว่า 1)

จากผลการศึกษาทั้งหมด ผลกระทบต่อระบบสืบพันธ์ในเพศชายมีแนวโน้มของความสัมพันธ์กับการสัมผัสในระยะยาว การศึกษาครั้งนี้แนะนำให้มีการส่งเสริมและให้ความรู้ในการใช้สารกำจัดศัตรูพืชและการป้องกันตัวเองอย่างเหมาะสม และแนะนำให้มีการพัฒนาโปรแกรมการลดความเสี่ยงจากการสัมผัสสารกำจัดศัตรูพืชโดยเพิ่มความระมัดระวังและตระหนักของเกษตรกรต่อผลกระทบสุขภาพที่อาจจะเกิดขึ้น

สาขาวิชา สาธารณสุขศาสตร์	ลายมือชื่อนิสิต ....
ปีการศึกษา 2560	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
	ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

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THANT ZAW LWIN: PESTICIDES EXPOSURE AND HEALTH RISK ASSESSMENT: A CASE STUDYOF HEALTH EFFECT ON MALE GROUND-NUT FARMERS' REPRODUCTIVE SYSTEM IN KYAUK-KAN VILLAGE OF NYAUNG-U DISTRICT, MANDALAY REGION, MYANMAR. ADVISOR: ASSOC. PROF. WATTASIT SIRIWONG, Ph.D., CO-ADVISOR: PROF. MARK G. ROBSON, Ph.D., 265 pp.

Exposure to pesticides affects many body organs including reproductive system. This is the first of its kind pioneering study in Myanmar and the main objective of the present study review was to explore knowledge and practice on safe use of pesticide among farmers and to find out the effects of pesticide on male reproductive system by using biomarkers (semen, serum hormone and blood cholinesterase level) among male farmers in Kyauk Kan village of Nyaung-U District, Mandalay Region, Myanmar and accompanied with nine specific objectives. There were 3 phases, the first phase was cross-sectional study named as observational study, identified the health problems related to pesticide exposure, explored knowledge and practice on safe use of pesticide among ground-nut farmers among both male and female (n=400) in the community by interviewing with questionnaires. Nearly one-third of the respondents were in 38 to 47 years age group. Half of the respondents in this study (53.5%) of the ground-nut farmers in this study have the moderate knowledge level and (79.2%) have poor practice level for pesticide utilization and in this phase I, most of participants of both male and female were difficult to follow the pesticide utilization and used over amount of pesticide instruction. And also there was no association between socio-demographic characteristics of the respondents with knowledge and practice level upon pesticide exposure protection. For the second phase (Phase II), was also a cross-sectional study and named as laboratory study, only 100 male ground-nut farmers who were randomly selected from phase I of male participants, found out the effect of chemicals on male reproductive function of ground-nut farmers who were chronically exposed especially to organophosphate pesticides by using biomarkers in growing and non-growing periods. The average age (±) SD of all was 37.51±9.45 years old and half of the respondents are primary education level. Wilcoxon signed rank test was used for comparing differences between growing and non-growing period among biomarkers and there were statistically significant at $_{p}$H, viscosity, motility, morphology and sperm count in Semen Analysis, changes at Follicle-stimulating hormone and Testosterone level in Serum Hormonal Analysis and in Blood Cholinesterase test, changes in Haemoglobin Adjusted Acetyl Cholinesterase (HAChE) and Plasma Cholinesterase (PChE) respectively and p-value were less than 0.05 level . Association between biomarkers related with pesticide exposure and work related factors at growing and non-growing period by binary logistic regression analysis and some factors were found with significant association levels (p value <0.05).All the findings of phase II provide further evidence that prolonged exposure to pesticides can cause illness if they are incorporated over a longer period, even if the amounts taken up are relatively small and reported to be associated with reproductive dysfunction by reducing brain acetyl-cholinesterase activity, thus impairing hypothalamic and/or pituitary endocrine functions and reduce the semen quality. Risk assessment was done in last phase (Phase III) by hand wipe test to assess pesticide residues from dermal exposure and the average daily dose (ADD) of ground-nut farmers at 3.66×10^{-5} mg/kg-day in growing period among randomly selected 30 samples of ground-nut farmers from Phase II. For non-carcinogenic risk characterization, Hazard Quotient (HQ) were used to estimate risk and male ground-nut farmers' hand at mean was 0.12 and it was less than the acceptable level (HQ ≤1), therefore, ground-nut farmers in this area might not get a higher risk from ground-nut consuming that contaminated with chlorpyrifos. In view of the above findings, male reproductive dysfunction seems to be associated with chronic pesticides exposures and suggestion of handling and practicing of pesticide use and personal protective equipment should be educated and develop pesticide risk reduction program for health promotion and prevention among the farmers to increase health awareness and health concern.

Field of Study: Public Health
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Student's Signature ______________
Advisor's Signature ______________
Co-Advisor's Signature ______________

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LISTS OF ABBREVIATIONS

AchE	Acetylcholinesterase Enzyme
ABS	Absorption factor
ADD	Average Daily Dose
AOAC	The Scientific Association Dedicated to AnalyticalExcellence®
AT	Average Time
BW	Body Weight
C	Pesticide Concentration
CA	Carbamate
ChE	Cholinesterase
ED	Exposure Duration
EF	Exposure Frequency
GC	Gas Chromatography
HAChE	Haemoglobin Adjusted Acetylcholinesterase Enzyme
HQ	Hazard Quotient
HRA	Health Risk Assessment
IRIS	US EPA’s Integrated Risk Information System
LOD	Limit of Detections
LOQ	Limit of Quantifications
OPs	Organophosphate Pesticides
PChE	Plasma Cholinesterase
PR	Pesticide Residue
PY	Pyrethroid
Ref;	Reference
RfD	Reference Dose
RME	Reasonable Maximum Exposure
US EPA	United State Environment Protection Agency

CHAPTER I
INTRODUCTION

1.1 Backgrounds and Rationale

For every people, fruits and vegetables are important components of the human diet which are required for most of the reactions occurring in the body. Like other crops, fruits and vegetables are attacked by pests and diseases during production and storage leading to damages that reduce the quality and the yield. In order to reduce the loss and maintain the management techniques during cropping to destroy pests and prevent diseases. Nowadays, people using of increase amount of pesticides because they have rapid action, decrease toxins produced by food infection organisms and are less labor intensive than other pest control methods. However, the use of pesticides during production often leads to the presence of pesticide residues in fruits and vegetables after harvest (1).

A pesticide is defined as any substance or mixture of substances intended for preventing, destroying or controlling any pest including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food agricultural commodities, wood and wood products or animal food-stuff or which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies (2).

In developing countries many agricultural hazards occur annually and acute as well as chronic intoxication of chemical pesticides still remains a public health problem. Human beings are now living in an environment in which at least 10,000 different chemicals are prevalent and to which some 700-1000 new compounds are added annually. Little is known about environmental chemicals might interact in subtle ways to affect reproduction. The majority of agricultural workers in developing countries is illiterate or with lower educational status that come from a rural or agricultural back ground and are neither trained to handle machine nor appraised of the hazards or risks involved. And facilities for training of agricultural workers are in adequate (3).

According to the World Health Organization, poisoning can be prevented if pesticides are used safely and proper precautions are taken. To overcome health problem of pesticides poisoning, a health promotion programme for safe use of pesticides was conducted for a group of voluntary farmers (4). Accidental exposure or high level exposure to pesticides can have serious health implications. The potential for pesticides accident is real. While most of these pesticides can be used with relatively lower risk as long as label directions are followed, some are extremely toxic and special precautions (5).

If human is exposed to many environmental agents that may harmful to his reproductive capacity(6). Giwercman A and Bonde JP (7) discovered that many industrial and agricultural activities that generated chemical and physical agents will become a great threat to the reproductive function of male if they are exposed to male. From an environmental point of view, male reproductive disorders that include: sexual dysfunction, infertility and testicular cancer(8). Farmers, using the pesticides without proper protective measures will have a great risk of exposures to the above mentioned disorders(9). Currently, scientists have well-noticed that man-made chemicals may have a great tendency to disrupt reproductive function in both wildlife and humans.

While pesticides are being used throughout the world, concerns about the effects on health can be found in certain number of reports. And activities to prevent such effects are carried out in most countries and at an international level. In developing world an estimated three millions severe pesticide annually, of which 220,000 are fatal. About 3% of agricultural workforce in developing countries is estimated to suffer some pesticide intoxication each year (10). Misusing and also over using of pesticides may occur undesired accidents. Moreover it can cause adverse effects on environment and non-targeted living organisms accidentally. No matter how pesticides are useful for human beings, they will always carry the risks (11).

Agriculture sector is one of the major players of Myanmar economy. Myanmar has been now trying to transform its agriculture based economy into agriculture based industrialized economy. It has a population round about 53 million in 2014 and a population growth rate of 1.29 %. At present of 70% of the total population resides in the rural areas. The agriculture workforce constitutes about 56.47 % of the entire workforce. The agriculture sector contributes 40% of the Gross Domestic Product

(GDP) and 47% of the foreign earnings of Myanmar(12). The need for the health care of agriculture workers is accentuated by the number of persons involved and their very significant contribution to the national, economy, thus the control of agricultural hazards is essential for the agricultural workers as well as their families (13).According to Myanmar agricultural statistics (2004 – 2005) pesticides utilized for plant protection by crops in Myanmar was 26,461 pounds and 33,716 gallons. Another study conducted by Toxics vigilance and Prevention of Poisoning Unit in 2003, showed that there were 413 pesticide poisoning cases in Myanmar and 43 deaths out of those (14). According to the local study on acute poisoning conducted from October 2002 to September 2003, 24.4% was belonging to the pesticides (15). Since majority of Myanmar agricultural labors are illiterate , they are unable to understand completely about the instructions of how to safely used pesticide Sometimes and many a time at least 2 to 3 different kinds of pesticides are needed to use together and sometimes up to 5 kinds are mixed and sprayed on the crops. These pesticides are used by the farmers without noticing any restrictions and precautions and also the empty containers are not disposed-off systemically. As a consequence, it has also a great negative impact on the land area. Training programmes on safe use of pesticides are not enough to cover the whole population of agricultural workers (16).

There are several reasons why reproductive and environmental epidemiologists have taken interest in male reproductive health. Improved working conditions in affluent countries have dramatically decreased known hazardous workplace exposures, but millions of workers in less affluent countries are at risk from reproductive toxicants (7). Prior research has indicated that there may be associations between exposure to pesticides of a variety of classes and decreased sperm health(17). Spermatogenic process is regulated by the male endocrine system (18), and therefore, semen quality may be particularly sensitive to any pesticides or pesticide metabolites that may mimic male hormones or inflict tissue damage in the testes. A number of pesticides or their metabolites have been implicated as potential endocrine disruptors in human or animal models, including DDT (19) and pyrethroids (17). Recent studies have found association between pesticides or metabolites and levels of reproductive hormones in various populations. Among male floriculture workers in Mexico, for example, increased occupational exposures to organophosphates, measured as dialkylphosphates in urine, were associated with decreased levels of follicle stimulating hormone (FSH), increased levels of testosterone and decreased levels of inhibin B (20). Organochlorines were shown to be associated with differences in hormone levels in a cohort study of European men (8). Animal studies have also demonstrated the potential for pesticides to cause testicular damage. Tissue damage in the testes and adverse spermatogenic effects have been seen in rats and mice exposed to cypermethrin (21), malathion (22), and parathion (23).

Until relatively recently, the possible role of external environment factors in male reproduction had only been studied in experimental animals. An increasing number of human studies in recent years have started to evaluate the potential of pesticides to affect sperm quality, one of many factors related to male-factor infertility. Between 1991 and 2006, 20 studies were published in which the outcomes of interest were common semen quality measures (24). The main problem of pesticide-related reproductive affects are increasing use of pesticides in less-developed countries, often in the absence of adequate safety precautions, might further jeopardize the reproductive health of populations with high rates of reproductive wastage and infertility and the other thing that the progressive penetration of pesticides with endocrine-disrupting potential into the environment (and *into* human tissues) might have fat-reaching effects on human health in the long run (25).

Since Myanmar is an agro-industrial country, the majority of country’s economic sector is agriculture. Majority of country's work force consists of agricultural workers. Pesticides poisoning is one of the agricultural hazards in our country. In Myanmar, there are many groups of pesticides that can be used. They are pesticides such as organochlorine, organophosphate, carbamate, fungicides, herbicides, fumigants, rodenticides, etc. Among of these, organophosphate groups, organochlorine groups and carbamate groups are used commonly. Farm workers can have or buy various kinds of pesticides in market today. Utilization of pesticides without safety precaution results in adverse effects on health. It is necessary to have insight to the knowledge and practice of farm workers in using pesticides. Although the pesticides are useful for human beings, they are hazardous in the health of human beings. Therefore, it is necessary to assess pesticide residues on human’s health effects. Pesticides can cause health hazards on human (26). Safe utilization of pesticides is very important to reduce harmful effect on agricultural workers. Health hazards of pesticides can be reduced by proper understanding of safety measures of pesticide utilization and practice of using personal protective equipment. It is essential to provide health education on utilization of safety measures among pesticide applicators including manufacturers, sellers and users. Lack of knowledge of farm workers on pesticides may lead to occurrence of pesticide poisoning and moreover deliberate used of pesticides may lead to present of residues on fruits and vegetables and it is also necessary to assess knowledge, attitude and practice of farm workers in pesticide usage.

This is the first of its kind pioneering study in Myanmar, the study is to criticize all the results which evaluated the pesticide effect on male reproductive system. Results from this study was applied in Mandalay Region to provide information on the knowledge of pesticides among agricultural workers. But hopefully that the results were applied as a basis for further more researchers regarding issues among pesticide usage services in agricultural workers.

1.2 Research Gap

There were no research on effect of pesticide exposures on semen characteristics and reproductive hormone concentration in male reproductive system among farmers in agricultural area of Myanmar.

1.3 Research Objectives

The main objective of this study is to explore knowledge and practice on safe use of pesticide among farmers and to find out the effects of pesticide on male reproductive system by using biomarkers among male farmers in Kyauk Kan village of Nyaung-U District, Mandalay Region, Myanmar.

Specific Objectives

1) To assess the knowledge and practice on safe use of pesticide among ground-nut farmers.
2) To identify favorable environment of safe use of pesticide among ground-nut farmers.
3) To find out the effect of chemicals on male reproductive function by determining the seminal profile of ground-nut growing male farmers who are chronically exposed to different kinds of pesticides.

4) To determine the blood hormonal level of ground-nut growing male farmers for finding out the effect of chemicals on male reproductive function who are chronically exposed to different kinds of pesticides.
5) To measure the biomarkers of pesticide exposure among the male farmers by blood cholinesterase monitoring in ground-nut farmers.
6) To assess the risk related to dermal exposure by using gauze patch samples in ground-nut farmers.
7) To compare seminal profile, blood hormone level and blood cholinesterase of ground-nut growing male farmers between growing and non-growing seasons.
8) To determine the association between socio demographic characteristics, knowledge, practice on safe use of pesticide among ground-nut farmers.
9) To provide suggestions to community on health risk and safety guidelines to reduce the risk from pesticide for ground-nut farmers.

1.4 Research Questions

1) Are the ground-nut farmers in Kyauk-Kan Village, Nyaung-U District, Mandalay Region, Myanmar getting risk related to pesticides exposure?
2) Is there a relationship between semen analysis and blood hormonal levels and pesticide exposure routes in ground-nut farmers' male reproductive system in Kyauk-Kan Village, Nyaung-U District, Mandalay Region, Myanmar?
3) Is there an association between health effects and pesticides exposure in the ground-nut farmers in Kyauk-Kan Village, Nyaung-U District, Mandalay Region, and Myanmar?

1.5 Research Hypothesis

1) Ground-nut farmers in Kyauk-Kan Village, Nyaung-U District, Mandalay Region, and Myanmar are at risk of pesticides exposure.
2) There is a relationship between semen analysis level, blood hormonal levels , blood cholinesterase level and pesticide exposure concentration in ground-nut farmers' male reproductive system in Kyauk-Kan Village, Nyaung-U District, Mandalay Region, and Myanmar.

3) There is an association between health effects especially for reproductive system and pesticides exposure in the ground-nut farmers in Kyauk-Kan Village, Nyaung-U District, Mandalay Region, Myanmar
4) There is a difference of semen and blood hormonal levels between growing and non-growing season in ground-nut farmers' male reproductive system in Kyauk-Kan Village.

1.6 Conceptual Framework

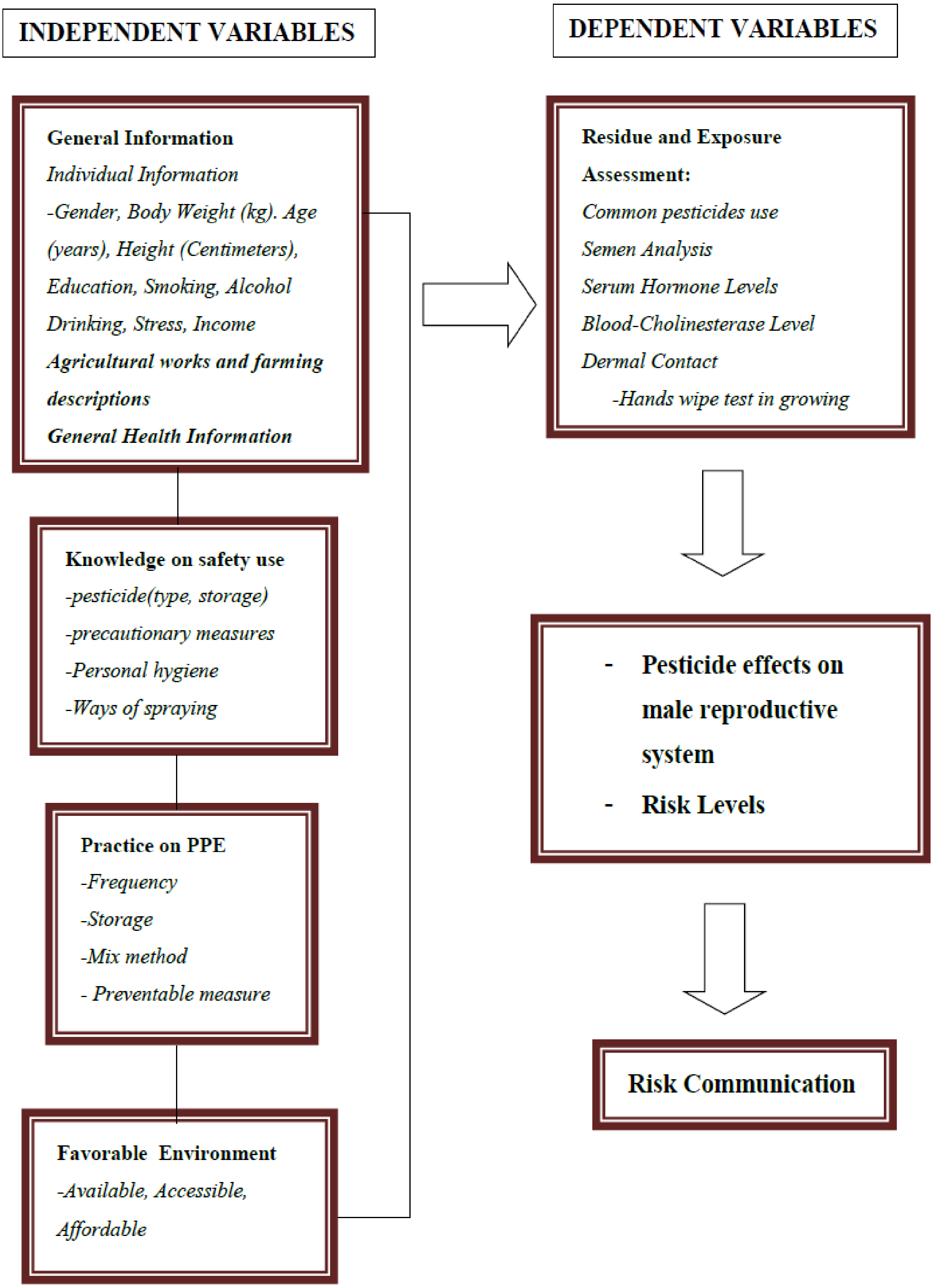

Figure 1.1 Conceptual Framework

1.7 Operational Definition

Farmer

In this study, farmer is a ground-nut farmer whose age between 18-49 years of reproductive age group, living in Kyauk-Kan village, Nyaung-U District, Mandalay Region, Myanmar more than 5 years. Normally they had to be pesticide applicator and engage all their farm activities.

Gender

For observational study, using questionnaires and assess upon knowledge upon pesticides and health effects and at that time, both male and female farm workers who are 18 to 49 years old of reproductive age group will include in this study but for laboratory study, to find out the pesticide effect on male reproductive function or not and so at that time, survey only on male and age also between 18 to 49 years of reproductive age group according to data from Health in Myanmar, Ministry of Health (27).

Duration of work

Total completed years of working duration of farm workers. According to inclusion criteria, living in the study area more than 5 years and reference year from one of the agricultural study of Myanmar(28).

Health Effects

Health effects of pesticides depend on the type of pesticide. For organophosphates affect on nervous system as well as skin and eyes symptoms. Some of pesticides may be carcinogens and some others may affect the hormone or endocrine system in the body(29). This study concentrates on the occupational exposure to pesticide residues among male ground-nut farmers based on finding of biomarkers test such as semen analysis, serum hormone level, blood cholinesterase level and assessing as dermal route for pesticide residues on hands. Effect or health symptoms from exposure to pesticide residues among male ground-nut farmers, which were collected by asking questionnaires, laboratory analysis and physical examination methods.

Pesticides and common pesticides use

It is the natural, biological or chemical substances used by agricultural workers to prevent or destroy insects, nematodes or weeds which destruct their plants. In

Myanmar, there are many groups of pesticides that can be used. There are pesticides such as organochlorine, organophosphate, carbamate are used commonly at Myanmar.

Agricultural works and farming descriptions

This is related with general information of agricultural and pesticide usage. The area, working years and tasks of farming are represented as the general information of agricultural. Pesticide usages include years of using pesticide, times of annual application and the equipment condition.

Use of personal protective equipment (PPE)

The use of personal protective equipment (PPE) is defined as the cover which ground-nut farmer use during the farm activities. It includes gloves, mask, goggles, boots, hat and coverall.

Residues of pesticides

The pesticide remaining on farmer's body is defined as residue of pesticide in this study. This study tries to find out the concentration of pesticides on reproductive system as hormonal assays and seminal analysis, on farmer's hands exposure assessment and using blood cholinesterase level in laboratory.

Exposure assessment

Exposure assessment is an evaluation of the potential exposures to human and the environment from the production, distribution, use, disposal and recycle of a chemical substance. In this study, exposure assessment is a process to estimate potential of pesticide exposure in ground-nut farmer in the study area via seminal analysis and hormonal assays method for male reproductive system assessment and dermal contact and biomarker. In generally, exposure assessment includes water and soil contact but in this study, just dermal contact was done.

Semen Analysis

Adverse effects of pesticides on the male reproductive system especially semen characteristics (semen volume level, viscosity , motility, morphology and sperm count) are an important health problem and have been conducted on causes of endocrine disrupters, one of the most famous of which are pesticides, showing evidence of reduction in semen quality due to agricultural pesticides.

Serum Hormones Analysis

Impairment of serum hormone levels has also been observed in organophosphate (OPs) exposed subjects. OPs are suspected to alter hormone levels by reducing brain acetylcholinesterase activity and impairing hypothalamic-pituitaryendocrine functions and gonadal process(30). This study concern for adverse human health risks resulting from exposure to environmental endocrine-disrupting compounds, in which men may be associated with or led to decline reproductive capacity or possibly increased risk of testicular function, forming infertility, prostate or thyroid cancer. A number of environmental chemicals may cause altered hormones levels through various biological mechanisms and target sites, ranging from effects on hormone receptors to effect on hormone synthesis, secretion or metabolism.

Dermal contact

In this study, dermal contact was done by hand wipe sample and it is defined as a method to collect the residue of pesticide on farmer’s hand for analyzing the contamination of pesticide residue on hands after a farmer finish his job task in farm followed by WHO method.

Blood Cholinesterase Level

The health effects of pesticide residue among the farm workers will be assessed by physical examination and by testing of level of Cholinesterase enzyme in whole blood. The blood cholinesterase test measures the effect of exposure to organophosphate and carbonate insecticides. Since cholinesterase levels can vary considerably among individuals, a “baseline” must be established for each person. In fact, a small percentage of the population has a genetically determined low level of cholinesterase. Even minimal exposure to cholinesterase inhibitors can present a substantial risk to these people (31).

Pesticide effects on male reproductive system

Some, such as the organophosphate pesticides, affect the nervous system and hypothalamo-gonadial system and effect on the spermatogenesis(32). This study concentrates on health effects of pesticide exposure on male reproductive system.

Risk Levels

It is the health risks and probability of health effects from occupational exposure to pesticides among ground-nut farmers based on health risk assessment and bio-markers (semen, serum hormone, blood cholinesterase) levels and dermal exposure assessment (path samples).

Risk Communication

Risk communication is very important to ensure public awareness of the hazard, methods for resolution, and public acceptance of these efforts. For risk communication, it was clearly mentioned that "an interactive process of exchange of information and opinion among individuals, groups and institutions. It involves multiple messages about the existence, nature, form, severity or acceptability of health risk". Risk communication plan must be sound, with effective strategies, monitoring and evaluation to ensure the desired objectives are achieved (33).

In the last part of this study includes risk communication. The results gave the information to the community by giving health education and the development of risk communication materials; hand books with communicated using PPE picture for encouraging ground-nut farmers to realize and concern their health, providing the knowledge of pesticide exposure to the farmer for themselves protection and also demonstration of personal protective equipment: how to wear the proper way and donation of PPE to the community.

1.8 Benefits of the study

1) The finding of the study provided increased agrochemical safety knowledge and behaviors resulting in preventing potential risks of agrochemicals among ground-nut farmers.
2) The development guideline was implemented for preventing and reducing exposure to pesticide residues on ground-nut farmers.
3) Policy implementation and risk communication can introduce to the community after finding the dermal exposure assessment of pesticide exposure in order to keep sustainable improvement of farmers in this community.

4) The findings of this study results and the guideline were applied to other farmer communities where use the similar pesticides and pattern of spraying pesticide for how to prevent from pesticide exposure and reduce the risk.

CHAPTER II
LITERATURE REVIEW

2.1 General Aspects/ Onset of Reproductive Life

Reproduction is essential for *perpetuation of species.* Reproduction has a homeostatic significance since it maintains a stable dynamic state in a particular species beyond its life span by generating new beings to take the place of the old and dying ones. In higher organisms like humans, there are two sexes involved – male and female explain in table 2.1.

Table 2.1 General aspects of Male vs Female in reproductive life

General characteristics	**Male**	**Female**
Genetic or chromosomal sex	44XY	44 XX
gonadal sex	testes	ovaries
genital sex	male genitalia	female genitalia
psychosocial sex	typical male behavior	typical female behavior

2.1.1 Physiologic Anatomy of the Male Sexual Organs

Figure 2.1-A shows the various portions of the male reproductive system, and Figure 2.1- B gives a more detailed structure of the testis and epididymis. The testis is composed of up to 900 coiled *seminiferous tubules*, each averaging more than one half meter long, in which the sperm are formed. The sperm then empty into the *epididymis*, another coiled tube about 6 meters long. The epididymis leads into the *vas deferens*, which enlarges into the *ampulla of the vas deferens* immediately before the vas enters the body of the *prostate gland.* Two *seminal vesicles*, one located on each side of the prostate, empty into the prostatic end of the ampulla, and the contents from both the ampulla and the seminal vesicles pass into an *ejaculatory duct* leading through the body of the prostate gland and then emptying into the *internal urethra. Prostatic ducts*, too, empty from the prostate gland into the ejaculatory duct and from there into the prostatic urethra. Finally, the *urethra* is the last connecting link from the testis to the exterior. The urethra is supplied with mucus derived from a large number of minute *urethral*

glands located along its entire extent and even more so from bilateral *bulbourethral glands* (Cowper's glands) located near the origin of the urethra (34).

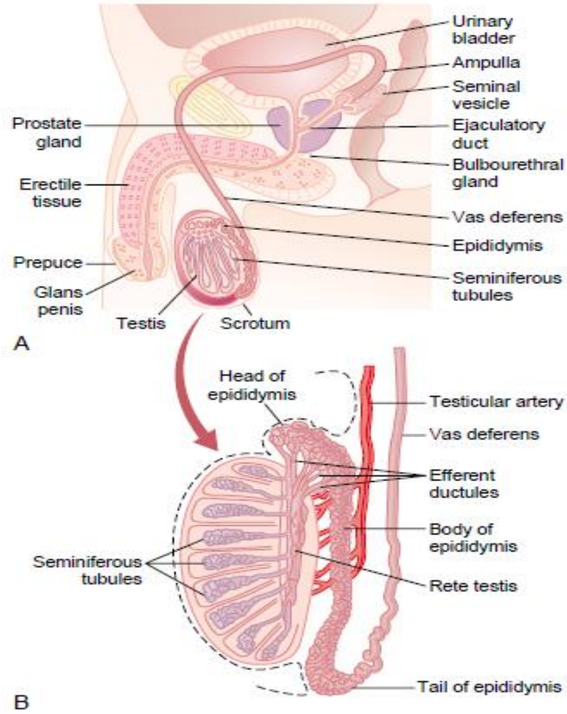

Figure 2.1 Physiologic Anatomy of the Male Sexual Organs
A, Male reproduction system (35) *B,* Internal structure of the testis and relation of the testis to the epididymis (36).

2.1.2 The Primary Sex Organs or Gonads

The primary sex organs essential for reproduction are called gonads. The *gonads* (a pair of testes in males and a pair of ovaries in females) have a dual function:

1. Gametogenesis : production of germ cells (gametes):
   Spermatogenesis in males – produces spermatozoa
   Oogenesis in females - produces ova.
2. Endocrine function – production of hormones that determine msleness or femaleness (sex hormones).
   Thus,
   - A pair of testes in males produces spermatozoa and male sex hormones, androgens.
   - A pair of ovaries in female produces ova and female sex hormones oestrogens (34).

2.1.3 Secondary Sex Organs (Genitalia)

Other sex organs necessary for the completion of the reproductive process and which depend on gonadal hormones for their full development and function are called secondary sex organs (e.g. prostate gland in males; uterus in females) (34).

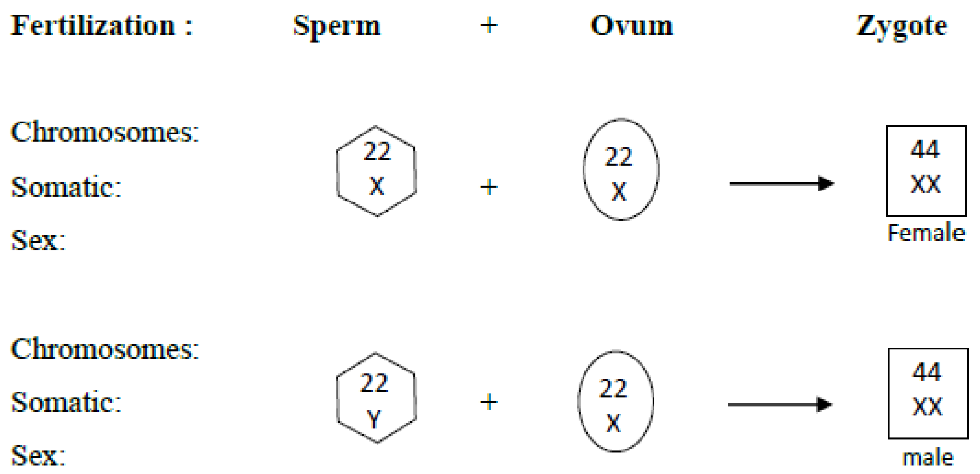

Figure 2. 2 Chromosomes in male and female

2.1.4 Reproductive Life (period)

In males – beings at puberty (10 -14 years of age) and remains reproductive till over age; characterized by continuous production of spermatozoa thus being fertile throughout the reproductive life.

In females – beings at puberty (9-13 years of age) and ends at **climacteric** when menstrual cycle ceases (**menopause:** around 45 -55 years of age). The female reproductive life is characterized during preganancy and lactation. Thus females are fertile only for a few days following each ovulation. Puberty is the period when the endocrine and gametogenetic functions of the gonads of both sexes have first developed to the point where *reproduction is possible.* In girls, the first observable event is the larch the development of breasts, followed by puberche**,** the development of axillary and pubic hair, and then by menarche**,** the first menstrual period. In boys, the first observable event is enlargement of testes and penis. Adolescence is the period from the earliest signs of puberty until the attainment of physical, mental and emotional maturity (34).

2.2 Male Reproductive System

2.2.1 Primary Sex Organs

A pair of testes:

1. Seminiferous tubular epithelium: Spermatogenesis – for fertility
2. Interstitial cells of Leyding.

   Production of androgens – for virility (masculinity)
3. Sertoli Cells (in seminiferous tubular epithelium): secretes:

2.2.1.1 Mullerian Regression Factor (Mullerian Inhibiting Substance, MIS)

1. Development of male internal genitalia during fetal life (it causes regression of the mullerian duct which would otherwise develop into female internal genitalia);
2. Testicular descent to the inguinal region.

   Inhibin (for feedback control of FSH)

   Oestrogens (small amounts) (35).

2.2.2 Secondary Sex Organs

Internal genitalia:

- Epididymis (to store spermatozoa) {together with vas deferences, ejaculatory duct, and penile urethra, forms the seminal tract for conveying semen to outside}
- Seminal vesicle
- Prostate gland
- Bulbourethral (Cowper's) glands

External genitalia:

- Peins – a copulatory organ by which semen is deposited in female genital tract
- Scrotum – a sac which encloses testes; functions as cooling mechanism for testes (35).

2.3 Spermatogenesis

During formation of the embryo, the *primordial germ cells* migrate into the testes and become immature germ cells called *spermatogonia* which lie in two or three layers of the inner surfaces of the *seminiferous tubules* (a cross section of one is shown in Figure 2.3-A.The spermatogonia begin to undergo mitotic division, beginning at puberty, and continually proliferate and differentiate through definite stages of development to form sperm, as shown in Figure 2.3-*B (34).*

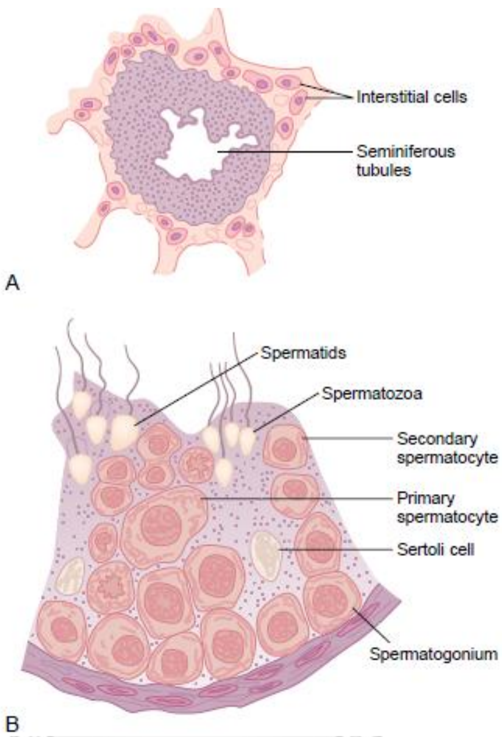

Figure 2.3 Spermatogenesis A, Cross section of a seminiferous tubule. B, Stages in the development of sperm from spermatogonia (36).

2.3.1 Steps of Spermatogenesis

Spermatogenesis occurs in the seminiferous tubules during active sexual life as the result of stimulation by anterior pituitary gonadotropic hormones, beginning at an average age of 13 years and continuing throughout most of the remainder of life but decreasing markedly in old age. In the first stage of spermatogenesis, the spermatogonia migrate among *Sertoli cells* toward the central lumen of the seminiferous tubule. The Sertoli cells are very large, with overflowing cytoplasmic envelopes that surround the developing spermatogonia all the way to the central lumen of the tubule.

Meiosis. Spermatogonia that cross the barrier into the Sertoli cell layer become progressively modified and enlarged to form large *primary spermatocytes* (Figure 2.4). Each of these, in turn, undergoes meiotic division to form two *secondary* *spermatocytes*. After another few days, these too divide to form *spermatids* that are eventually modified to become *spermatozoa* (sperm). During the change from the spermatocyte stage to the spermatid stage, the 46 chromosomes (23 pairs of chromosomes) of the spermatocyte are divided, so that 23 chromosomes go to one spermatid and the other 23 to the second spermatid. This also divides the chromosomal genes so that only one half of the genetic characteristics of the eventual fetus are provided by the father, while the other half are derived from the oocyte provided by the mother.

The entire period of spermatogenesis, from spermatogonia to spermatozoa, takes about 74 days during embryonic development the primordial germ cells migrate to the testis where they become spermatogonia. At puberty (usually 12 to14 years after birth), the spermatogonia proliferate rapidly by mitosis. Some begin meiosis to become primary spermatocytes and continue through meiotic division I to become secondary spermatocytes. After completion of meiotic division II, the secondary spermatocytes produce spermatids, which differentiate to form spermatozoa.

Sex Chromosomes. In each spermatogonium, one of the 23 pairs of chromosomes carries the genetic information that determines the sex of each eventual offspring. This pair is composed of one X chromosome, which is called the *female chromosome*, and one Y chromosome, the *male chromosome*. During meiotic division, the male Y chromosome goes to one spermatid that then becomes a *male sperm*, and the female X chromosome goes to another spermatid that becomes *female sperm*. The sex of the eventual offspring is determined by which of these two types of sperm fertilizes the ovum.

Formation of Sperm. When the spermatids are first formed, they still have the usual characteristics of epithelioid cells, but soon they begin to differentiate and elongate into spermatozoa. Each spermatozoon is composed of a *head* and a *tail*. The head comprises the condensed nucleus of the cell with only a thin cytoplasmic and cell membrane layer around its surface. On the outside of the anterior two thirds of the head is a thick cap called the *acrosome* that is formed mainly from the Golgi apparatus. This contains a number of enzymes similar to those found in lysosomes of the typical cell, including *hyaluronidase* (which can digest proteoglycan filaments of tissues) and powerful *proteolytic enzymes* (which can digest proteins). These enzymes play important roles in allowing the sperm to enter the ovum and fertilize it. The tail of the sperm, called the *flagellum*, has three major components: (1) a central skeleton constructed of 11 microtubules, collectively called the *axoneme*— the structure of this is similar to that of cilia found on the surfaces of other types of cells described in Chapter 2; (2) a thin cell membrane covering the axoneme; and (3) a collection of mitochondria surrounding the axoneme in the proximal portion of the tail (called the *body of the tail*). Back-and-forth movement of the tail (flagellar movement) provides motility for the sperm. This movement results from a rhythmical longitudinal sliding motion between the anterior and posterior tubules that make up the axoneme. The energy for this process is supplied in the form of adenosine triphosphate that is synthesized by the mitochondria in the body of the tail. Normal sperm move in a fluid medium at a velocity of 1 to 4 mm/min. This allows them to move through the female genital tract in quest of the ovum (37).

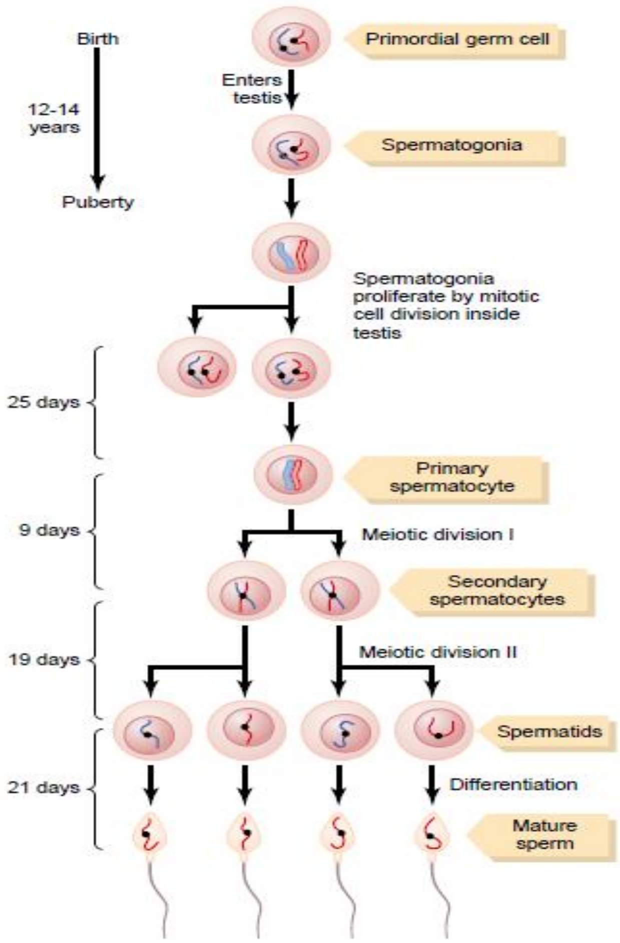

Figure 2.4 Cell divisions during spermatogenesis

2.3.2 Hormonal Factors That Stimulate Spermatogenesis

1. *Testosterone*, secreted by the *Leydig cells* located in the interstitium of the testis, is essential for growth and division of the testicular germinal cells, which is the first stage in forming sperm.

2. *Luteinizing hormone*, secreted by the anterior pituitary gland, stimulates the Leydig cells to secrete testosterone.

3. *Follicle-stimulating hormone*, also secreted by the anterior pituitary gland, stimulates the *Sertoli cells*; without this stimulation, the conversion of the spermatids to sperm (the process of spermiogenesis) will not occur.

4. *Estrogens*, formed from testosterone by the Sertoli cells when they are stimulated by folliclestimulating hormone, are probably also essential for spermiogenesis.

5. *Growth hormone* (as well as most of the other body hormones) is necessary for controlling background metabolic functions of the testes. Growth hormone specifically promotes early division of the spermatogonia themselves; in its absence, as in pituitary dwarfs, spermatogenesis is severely deficient or absent, thus causing infertility.

2.4 Testosterone and Other Male Sex Hormones: Secretion, Metabolism, and Chemistry of the Male Sex Hormone

2.4.1 Secretion of Testosterone by the Interstitial Cells of Leydig in the Testes

The testes secrete several male sex hormones, which are collectively called *androgens*, including *testosterone*, *dihydrotestosterone*, and *androstenedione*. Testosterone is so much more abundant than the others that one can consider it to be the significant testicular hormone, although as we shall see, much, if not most, of the testosterone is eventually converted into the more active hormone dihydrotestosterone in the target tissues. Testosterone is formed by the *interstitial cells of Leydig*, which lie in the interstices between the seminiferous tubules and constitute about 20 per cent of the mass of the adult testes, as shown in Figure 2.5.

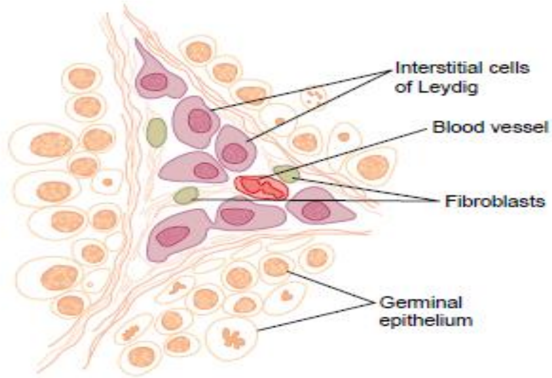

Figure 2.5 Leydig cell

Leydig cells are almost nonexistent in the testes during childhood when the testes secrete almost no testosterone, but they *are* numerous in the newborn male infant for the first few months of life and in the adult male any time after puberty; at both these times the testes secrete large quantities of testosterone. Furthermore, when tumors develop from the interstitial cells of Leydig, great quantities of testosterone are secreted. Finally, when the germinal epithelium of the testes is destroyed by x-ray treatment or excessive heat, the Leydig cells, which are less easily destroyed, often continue to produce testosterone (37).

2.4.2 Metabolism of Testosterone

After secretion by the testes, about 97 per cent of the testosterone becomes either loosely bound with plasma albumin or more tightly bound with a beta globulin called *sex hormone–binding globulin* and circulates in the blood in these states for 30 minutes to several hours. By that time, the testosterone either is transferred to the tissues or is degraded into inactive products that are subsequently excreted. Much of the testosterone that becomes fixed to the tissues is converted within the tissue cells to *dihydrotestosterone*, especially in certain target organs such as the prostate gland in the adult and the external genitalia of the male fetus. Some actions of testosterone are dependent on this conversion, whereas other actions are not (38).

2.5 Control of Male Sexual Functions by Hormones from the Hypothalamus and Anterior Pituitary Gland

A major share of the control of sexual functions in both the male and the female begins with secretion of *gonadotropin-releasing hormone (GnRH)* by the hypothalamus (see Figure 80–10). This hormone in turn stimulates the anterior pituitary gland to secrete two other hormones called *gonadotropic hormones*: (1) *luteinizing hormone (LH)* and (2) *follicle-stimulating hormone (FSH)*. In turn, LH is the primary stimulus for the secretion of testosterone by the testes, and FSH mainly stimulates spermatogenesis (39).

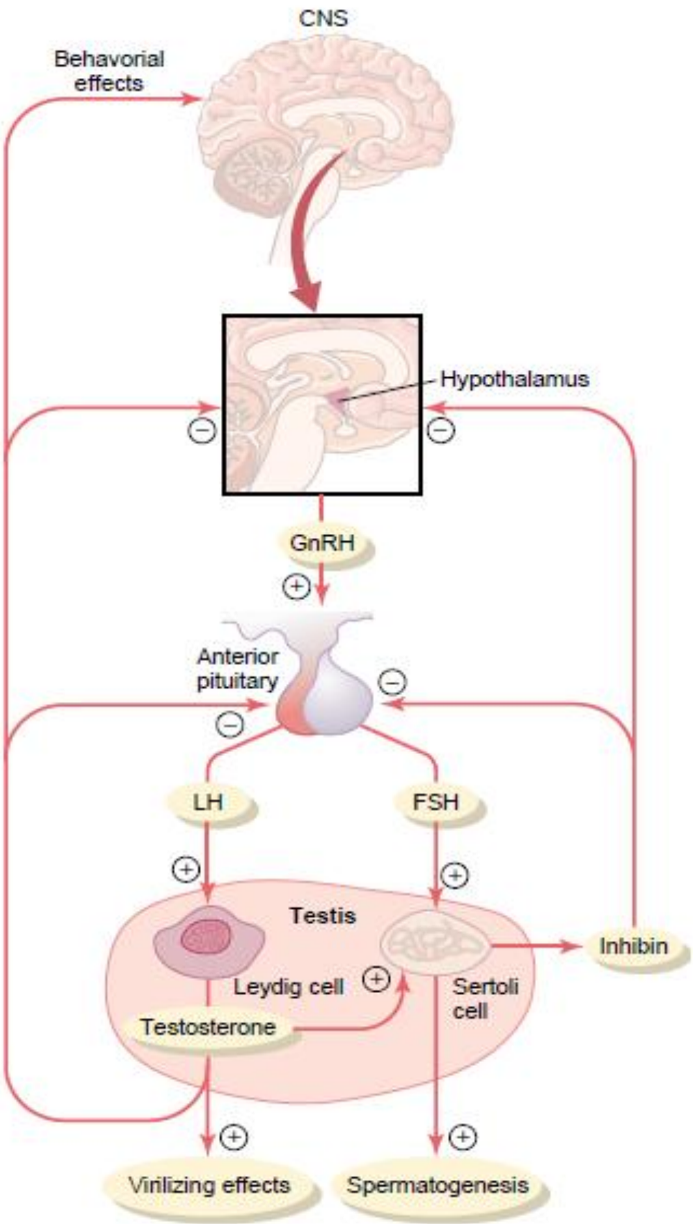

Figure 2. 6 Feedback regulation of the hypothalamic-pituitary-testicular axis in males.

2.5.1 GnRH and Its Effect in Increasing the Secretion of LH and FSH

GnRH is a 10-amino acid peptide secreted by neurons whose cell bodies are located in the *arcuate nuclei of the hypothalamus*. The endings of these neurons terminate mainly in the median eminence of the hypothalamus, where they release GnRH into the hypothalamic-hypophysial portal vascular system. Then the GnRH is transported to the anterior pituitary gland in the hypophysial portal blood and stimulates the release of the two gonadotropins, LH and FSH. GnRH is secreted intermittently a few minutes at a time once every 1 to 3 hours. The intensity of this hormone's stimulus is determined in two ways: (1) by the frequency of these cycles of secretion and (2) by the quantity of GnRH released with each cycle.The secretion of LH by the anterior pituitary gland is also cyclical, with LH following fairly faithfully the pulsatile release of GnRH. Conversely, FSH secretion increases and decreases only slightly with each fluctuation of GnRH secretion; instead, it changes more slowly over a period of many hours in response to longer-term changes in GnRH. Because of the much closer relation between GnRH secretion and LH secretion, GnRH is also widely known as LH releasing hormone *(32).*

Gonadotropic Hormones: LH and FSH: Both of the gonadotropic hormones, LH and FSH, are secreted by the same cells, called *gonadotropes,* in the secretion from the hypothalamus, the gonadotropes in the pituitary gland secrete almost no LH or FSH. LH and FSH are *glycoproteins*. They exert their effects on their target tissues in the testes mainly by *activating the cyclic adenosine monophosphate second messenger system*, which in turn activates specific enzyme systems in the respective target cells (32).

Testosterone Regulation of Its Production by LH. *Testosterone* is secreted by the *interstitial cells of Leydig* in the testes, but only when they are stimulated by LH from the anterior pituitary gland. Furthermore, the quantity of testosterone secreted increases approximately in direct proportion to the amount of LH available. Mature Leydig cells are normally found in a child's testes for a few weeks after birth but then disappear until after the age of about 10 years. However, either injection of purified LH into a child at any age or secretion of LH at puberty causes testicular interstitial cells that look like fibroblasts to evolve into functioning Leydig cells (39).

Inhibition of Anterior Pituitary Secretion of LH and FSH by Testosterone Negative Feedback Control of Testosterone Secretion: The testosterone secreted by the testes in response to LH has the reciprocal effect of inhibiting anterior pituitary secretion of LH (see Figure 80–10). Most of this inhibition probably results from a direct effect of testosterone on the hypothalamus to decrease the secretion of GnRH. This in turn causes a corresponding decrease in secretion of both LH and FSH by the anterior pituitary, and the decrease in LH reduces the secretion of testosterone by the testes. Thus, whenever secretion of testosterone becomes too great, this automatic negative feedback effect, operating through the hypothalamus and anterior pituitary gland, reduces the testosterone secretion back toward the desired operating level. Conversely, too little testosterone allows the hypothalamus to secrete large amounts of GnRH, with a corresponding increase in anterior pituitary LH and FSH secretion and consequent increase in testicular testosterone secretion (32).

Regulation of Spermatogenesis by FSH and Testosterone: FSH binds with specific FSH receptors attached to the Sertoli cells in the seminiferous tubules. This causes these cells to grow and secrete various spermatogenic substances. Simultaneously, testosterone (and dihydrotestosterone) diffusing into the seminiferous tubules from the Leydig cells in the interstitial spaces also has a strong tropic effect on spermatogenesis. Thus, to initiate spermatogenesis, both FSH and testosterone are necessary.

Negative Feedback Control of Seminiferous Tubule Activity— Role of the Hormone *Inhibin*: When the seminiferous tubules fail to produce sperm, secretion of FSH by the anterior pituitary gland increases markedly. Conversely, when spermatogenesis proceeds too rapidly, pituitary secretion of FSH diminishes. The cause of this negative feedback effect on the anterior pituitary is believed to be secretion by the Sertoli cells of still another hormone called *inhibin* (see Figure 80–10). This hormone has a strong direct effect on the anterior pituitary gland to inhibit the secretion of FSH and possibly a slight effect on the hypothalamus to inhibit secretion of GnRH. Inhibin is a glycoprotein, like both LH and FSH, having a molecular weight between 10,000 and 30,000. It has been isolated from cultured Sertoli cells. Its potent inhibitory feedback effect on the anterior pitu-itary gland provides an important negative feedback mechanism for control of spermatogenesis, operating simultaneously with and in parallel to the negative feedback mechanism for control of testosterone secretion (40).

2.6 Human Semen (Seminal Fluid)

It is a fluid ejaculated at the time of orgasm in the male and consists of cells (spermatozoa) and fluid (seminal plasma) which acts as an activator and diluent for spermatozoa.

2.6.1 Characteristics of Ejaculated Human Semen

1. Volume : 2 to 5 ml (about 1 teaspoonful) per ejaculation after 3 days Abstinence from sexual activity
2. Color : white opalescent (yellow sometimes)
3. Coagulation : immediate
4. Liquefaction : complete within 15 -30 min after ejaculation.
5. $_{p}$H : alkaline (7.2 -7.8)
6. Sperm count(millions per ml): 100 (range : 50 -200) (Lower limit of fertility: 20)
7. Sperm Motility (within 3 hr) : 60 -70 % actively motile
8. Sperm morphology : normal forms 80% or more

Examination of the above characteristics of semen (semenalysis) is done to assess male fertility (34).

2.6.2 Chemical constituents of Seminal Fluid

1. From seminal vesicles:

   Fructose: source of energy for sperms

   Flavins : impart yellow coloration

   Fibrinogen, thromboplastin : coagultion of semen

   Prostaglandins or function (and also or Uterine contractions for sperm transport).

2. From prostate gland

   Spermine: odour

   Fibrinogen,fibrinogenase : liquefaction

3. From bulbourethral glands mucus: for lubrication

   (also present in preejaculatory fluid)

4. From spermatozoa:

Hyaluronidase : for breadown of mucus barriers (34)

2.6.3 Control of Testicular Function

Testicular function is controlled by gonadotrophins and GnRH along the hypothalamo – adenohypophyseal – testicular axis with testicular hormones exerting negative feedback at both the pituitary and hypothalamic levels.

Hypothalmic control is in turn influenced by a number of neural and hormonal inputs. Hypothalamic lesions in humans lead to atrophy of the testes and loss of their function.

I. Control of Spermatogenic Function

(also refer to hormonal factors influencing spermatogenesis,)

Since FSH and androgens (principally testosterone) maintain spermatogenesis, the spermatogenic function of the tests is controlled by:

1. FSH along the cerebro – hypothalamo – hypophyseal – testicular (Sertoli cell) axis with

   Inhibin (a polypeptide secreted by Sertoil cells) exerting negative feedback control at the pituitary level.,i.e., FSH stimulates Sertoli cell activity which promotes spermatogenesis; the concurrent increase in circulating inhibit pituitary secretion of FSH.

   Inhibins are dimmers containing α and β subunits. Dimers containing subunits β only stimulate rather than inhibit FSH secretion and hence are called activins. Their exact role in the control of spermatogenic function is unsettled (32).

2. LH through testosterone along the cerebro – hypothalamo – hypophyseal – testicular (Leydig cell) axis.

   In response to LH, some of the testosterone secreted from the Leydig cells bathes the seminiferous epithelium and provides high local concentration of androgen necessary for normal spermatogenesis. Systemically administered testosterone does not raise the androgen level in the testes to as great a degree and it inhibits LH secretion. The net effect, generally, is a fall in endogenously produced testosterone and a resultant decrease in sperm count. Testosterone therapy has been suggested as a means of male contraception. However, the does needed to suppress spermatogenesis causes sodium and water retention. The possible use of inhibit as a male contraceptive is now being explored. The estrogens from the testes might also play a role in the regulation of FSH secetion since they have an inhibitory effect on FSH secretion (39).

II. Control of Endocrine Function

LH is trophic to Leydig cells. It stimulates the secretion of testosterone, which in turn feedbacks to inhibit LH secretion. Thus the endocrine function of testes is controlled by LH along the cerebro – hypothalamo – hypopseal – Leydig cell axis with testosterone exerting negative feedback control primarily at the hypothalamus level. Implantation of minute amounts of testosterone in the hypothalamus but not in the pituitary causes testicular atrophy, indicating that the feedback effect of testosterone on gonadotrophin secretion is at the level of hypothalamus (38).

Steroid Feedback: Castration is followed by a rise in the pituitary content and secretion of FSH and LH, and hypothalamic lesions prevent this rise. Estrogens lower the plasma testerone levels, presumably because they inhibit LH secretion (32).

2.7 Brief history of pesticides

Pesticides include a variety of chemical compounds used mainly in the agricultural sector and health sector to reduce losses from pests that attack cops and against insect vectors.Human being used pesticides many years ago. Ancient Romans killed pest by burning sulphur and controlled weed with salt. Human controlled ants with honey and arsenic in the 1600s. Farmers in United States of America also used copper acetoarsenite (Paris green), nicotine sulphate and sulphur as pesticides in late nineteenth century (11).

During the Second World War, the insecticidal potential of dichlorodiphenyltrichloroethane was discovered in Switzerland, and insecticidal organophosphorus compounds were developed in Germany. At about the same time, herbicides of the phenoxyalkonoic acid group was produced in Britain. In 1945, the first soil-acting carbamate herbicides were discovered by British workers, and the organochlorine insecticides chlordane was introduced in U.S.A and Germany. Shortly afterwards, the insecticides carbamates were developed in Switzerland. In 1950-5, the herbicidal urea derivatives were developed in the U.S.A, the fungicides captan and glyodin appeared, and malathion was introduced. Between 1955 and 1960, newcomers include herbicidal triazines and quartenary ammonium herbicides. Dichlobenil, trifluralin, and bromoxynil were described in 1960-5, and the systemic fungicide benomyl in 1968. The leaf-acting herbicide glyphosate was introduced soon afterwards (41).

Pesticides are substances that are used intentionally in agriculture, forestry, and horticulture and on public lands and in gardens to increase crop yields, improve the appearance of plant products, or to facilitate the care of open spaces. They are also referred to as plant protection products. In Europe, pesticides used outside of agriculture are called biocides (42). Biocides are used, for example in private households, to destroy unwanted or detrimental organisms and are also applied in large quantities in many developing countries to combat pathogenic organisms or species that serve as vectors (carriers) for pathogens i.e mosquitoes that are carriers of pathogens that cause malaria (43). All populations are assumed to be exposed to pesticides. The several of these substances is identified by data on contamination of food as well as surface, ground, and drinking water. In many parts of the world, low-level poisoning due to pesticide contamination of food poses a risk of chronic illness and adverse health effects on human beings. In developing countries, the effects of acute poisoning due to exposure to dangerous levels of pesticides in food are apparently more severe than in industrialized countries. In some regions, pesticide is used through direct contact in agriculture through direct contact is a problem. Mixing and applying pesticides can cause acute poisoning via the respiratory organs or through direct contact with the skin or eyes (44). Asia and the Pacific has conducted research that shows that 82 of the 150 pesticides used in Asia, including seven of the ten most used ones, are on the list of highly hazardous pesticides published by PAN (45).

2.8 Global Use of pesticides

In the early 1960’s, the debate about pesticides was largely confined to the industrialized nations. Today, however, pesticides are produced and used much more globally. Reasons of increasing trend of use of pesticides in the third world are:

(1) The high yielding crops were more susceptible to some pests and diseases than the native adapted species and were grown in monoculture rather than in diverse mixes of varieties and crops. By contrast, the traditional local varieties had evolved resistance to most of their local pests and diseases. Thus, it becomes necessary to use more pesticides to maintain the yield advantage conferred by the new varieties.
(2) Pesticides use in lesser developed countries also has increase because they are increasingly growing fruits and vegetables for sale to more developed countries, such as the US. They get high enough prices for these products to make pesticides purchases possible. In addition, consumers in most northern markets, such as the US, have high cosmetic standards for fruits and vegetables, necessitating that the crops be well-protected from pests and diseases. This trend towards more fruits and vegetables grown in lesser developed nations being marketed in northern countries have led to concerns about pesticide residues in imported produce.
(3) A third reason for the increase in pesticide use in less developed countries related to the changed growing conditions brought about by use of green revolution varieties and technologies. Beyond the monocultures discussed above, increase in irrigation and fertilization often improve conditions for pests, necessitating more control efforts.

The global distribution of pesticide use is as follow:

- North America uses about 30% of the world total
- Europe uses about 27%
- Japan uses about 12%
- Developing nations uses about 31% (46)

Environmental damage has become a global issue and Myanmar cannot remain divorced from these realities However, because of budgetary and foreign exchange constraints, the sum total of pesticide and fertilizer use does not meet the actual requirement which may be a blessing in disguise. As yet, pollution and contamination are not grave problems in Myanmar. The utilization of pesticides and fertilizers is very low compared to neighboring countries. Myanmar is one of the lowest fertilizer consumption countries in the Asia-Pacific Region (47).

2.9 Health Hazards due to pesticides

Pesticide poisonings are classified as both suicidal or intentional poisonings and unintentional poisonings. Unintentional poisonings can be resulted from accidents on the job or accidents outside of occupational contexts. Typical symptoms of poisoning in humans that are relatively easy to diagnose as acute pesticide poisoning. Typical symptoms of acute pesticide poisoning are fatigue, headaches and body aches, skin discomfort, skin rashes, poor concentration, feelings of weakness, circulatory problems, dizziness, nausea, vomiting, excessive sweating, impaired vision, tremors, panic attacks, cramps, etc., and in severe cases coma and death. Pesticides can also cause chronic illnesses if they are incorporated over a longer period, even if the amounts taken up are relatively small. Symptoms are often diffuse or do not become apparent for a long time, which then leads to late effects. Farm workers are especially at risk. Furthermore, pesticides can damage the human nervous system. There are indications that there is a connection between pesticide exposure and reduced sensitive faculties, disruptions in cognitive and psychomotoric functions and depression (48).

The WHO estimated that 849,000 people die globally from self-harm each year based on 2001 data (World Health Report, 2004). However, poisoning is the commonest form of fatal self-harm in rural Asia, accounting for over 60% of all deaths (49)and is of far greater importance than hanging and other physical forms of self-harm. Furthermore, a review of poisoning studies revealed that pesticides are the commonest means of self-poisoning in many rural areas and associated with a high mortality rate (50). A recent national survey in Bangladesh showed that 14% of all deaths (3971 of 28,998) of women between 10 and 50years of age were due to self-poisoning, the majority with pesticides (51).

Most harmful chemical pesticides are Organochlorine, Organophosphate, Carbamate, Phenoxy aliphatic acid, Bipyridyl, Poly chlorinated bi phenyl, Dibenzo furan and Chlorinated Di benzo-p- dioxin. DDT (Dichlorodiphenyl trichloroethane), Aldrine, Chlordane, Dieldrine, Heptachlor and Toxaphene etc. are organochlorinated compounds used as pesticides. These chemicals are persistent organic pollutant pesticides and can cause toxicity to natural eco systems. DDT has been shown to have teratogenic and carcinogenic effect in laboratory studies of animals. It also produces tremors and acute central nervous system toxicity in human. Chlordane is chlorinated cyclodienes and it can produce acute toxicity in human at high doses. It can also produce liver toxicity and cancer in mice. This chemical produces stupor and seizures and ingestion of small amount can cause death. Organophosphrous pesticides include Malathion, Parathion, Diazenon etc. Organphosphates are well absorbed by the skin, conjunctiva, gastrointestinal tract and the lungs. These compounds irreversibly inhibit the acetyl cholinesterase and cause diarrhoea, salivation, airway obstruction, restlessness, anxiety, miosis and muscle fasciculation. High dose can cause convulsion, coma and death by respiratory failure. It can also cause neuro toxicity. Carbamate pesticides include Carbaryl, Aldicarb and Thiodicarb etc. They are derivatives of carbamic acid and can produce reversible inhibition on acetylholinesterase. Toxicity of carbamate is similar to organophosphate but less potent and cause neurotoxicity (52).

A survey of health and the characterization of pesticide appliers in Minnesota were done in 1949 to know more about the pesticide use and its potential health effects. State Licensed pesticide appliers 1,000 in number were randomly selected. Participants were stratified by pesticide class (herbicides, insecticides, fungicides, and fumigant) to determine potential difference in health characteristics among different pesticide groups. A subset of 60 applicators, divided by pesticides class used was studied for exposure related cholinesterase depression. Cholinesterase depression in excess of 20% was most frequent in fumigant applicators that did enclose space application procedures ($p < 0.05$). Survey data demonstrated that the prevalence of all common chronic disease considered together was significantly increased ($p = 0.015$) in fumigant appliers, compared with all other pesticides use groups. The frequency of chronic lung disease was also significantly increased in fumigant applier group ($p = 0.027$) (53).

The study done at Inlay area (1995) reported that they conducted a cross sectional survey on 493 gardeners of floating vegetable gardens and residents from seven villages of Inlay Lake by using the questionnaire interview method, medical examination, plasma cholinesterase estimation sampling and analysis of pesticide residue in water, water weed and bottom sediments as they are exposed to a variety of agrochemicals. The prominent symptoms related to muscarinic action of organophosphorus insecticide poisoning such as sweating, hypersalivation and increased body temperature were found to be statistically significant ($p <0.05$). Plasma cholinesterase was found to be lower than the reference value in (120) individuals. Females had significantly lower plasma cholinesterase level than males ($p < 0.05$). Organochlorine type of pesticide residue was detected in water, water weed and bottom sediments. This study indicated a sub clinical intoxication of pesticides among gardeners of floating vegetable gardens and residents of Inlay Lake and the possible environmental pollution of the Inlay lake area (26).

2.10 Organophosphate Pesticide

Most organophosphate pesticides (OPs) are ester or thiol derivatives of phosphoric acid. General structure themselves is shown in Figure 2.1 R1 and R2 are alkyl groups, which are able to directly attach phosphorous atom or via an oxygen atom, or a sulfur atom. In some cases, R1 is directly bonded with phosphorous atom and R2 bonds with an oxygen or sulfur atom. The X group can be various and may belong to a wide range of aliphatic, aromatic or heterocyclic groups. The X group, which is known as a leaving group due to hydrolysis of the ester bond reaction, it will be moved out from phosphorous (54). Almost all of OPPs are slightly dissolved in water, have high oil to water partition coefficient, and low vapor pressure.

Organophosphates and carbamates have very different chemical structures, but share a similar mechanism of action and will be examined here as one class of insecticides. Organophosphates were initially developed in the 1940s as highly toxic biological warfare agents (nerve gases). When the organophosphate parathion was first used as a replacement for DDT, it was believed to be better as it was more specific. Unfortunately there were a number of human deaths because workers failed to appreciate the fact that parathion's short-term (acute) toxicity is greater than DDT's. The problem with organophosphates and carbamates is that they affect an important neurotransmitter common to both insects and mammals. This neurotransmitter, acetylcholine, is essential for nerve cells to be able to communicate with each other. Acetylcholine released by one nerve cell initiates communication with another nerve cell, but that stimulation must eventually be stopped. To stop the communication, acetylcholine is removed from the area around the nerve cells, and an enzyme, acetylcholinesterase, breaks down the acetylcholine. Organophosphates and carbamates block the enzyme and disrupt the proper functioning of the nerve cells. Hence, these insecticides are called acetylcholinesterase inhibitors. Structural differences between the various organophosphates and carbamates affect the efficiency and degree to which the acetylcholinesterase is blocked. Nerve gases are highly efficient and permanently block acetylcholinesterase, while the commonly used pesticides block acetylcholinesterase only temporarily. The toxicity of these pesticides presents significant health hazards, and researchers continue to work to develop new insecticides that have fewer unintended consequences (55).

(a) Organophosphate (b) Carbamate

Figure 2. 7 The general structure of organophosphate and carbamate pesticide form

History of organophosphate ester insecticides were first synthesized in 1973 by a group of German chemist led by Gerhard Schrader at Farbenfabriken Bayer AG. Many of their trial compounds proved to be exceedingly toxic and unfortunately, under the management of the Nazis in World War II, some were developed as potential chemical warfare agents. Although it is true that all of the organophosphate esters were derived from nerve gases, (chemicals such as soman, sarin and tabun) . The insecticides used today are at least three or more generations of development away from those highly toxic chemicals. The first organophosphate ester insecticides to be used commercially was tetraethyl-pyrophosphate (TEPP) and, although effective, it was extremely toxic to all forms of life and chemical stability was a major problem in that T.E.P.P hydrolyzed readily in the present of moisture. Further development was directed toward the synthesis of more stable chemicals having moderate environmental persistence. The organophosphate seems likely to continue to be the most important type of insecticides used in developing countries (29).

Mode of action of organophosphate insecticides elicits their toxicity by inhibition of the nervous tissue actelylcholoine esterase, the enzyme responsible for the destruction and termination of the biologic activity of neurotransmitter acetylcholine. With the accumulation of free and unbound acetylcholine at the nerve endings of all cholinergic nerves, there is continual stimulation of electric activity. The sign of toxicity include those resulting from stimulation of muscarinic receptors of the parasympathetic autonomic nervous system such as increased secretion, bronchoconstriction, miosis, gastrointestinal cramps, diarrhea, urination, bradycardia; those resulting from stimulation and subsequent blockade of nicotinic receptors causing tachycardia, hypertension, muscle fasciculation, tremors, muscle weakness and/or flaccid paralysis; and those resulting from CNS such as restlessness, emotional lability, ataxia, lethargy, mental confusion, loss of memory, generalized weakness, convulsion, cyanosis, coma (29).

2.10.1 Organophosphates effect on reproductive system

Animal studies have shown that organophosphates may decrease sperm density and motility and consequently, the fertility (56). Histological findings indicated enlargement of interstitial space, inhibition of spermatogenesis, rarefaction of Leydig cells and edema in testes in contact with them. In the animals exposed to methyl parathion, a decrease body and testis weights, a significant decrease in sperm counts and sperm motility, and an increase in abnormal sperm morphology were noticed. Necrosis and edema were also noted in the seminiferous tubules and interstitial tissues after 4 and 7 weeks of methyl parathion exposure (57). In another study, malathion reduced the weight of testes, epididymis, seminal vesicle, and ventral prostate in male Wistar rats. Testicular and epididymal sperm density were also reduced in these animals. Malathion also decreased the level of testosterone (58).

Another survey revealed that the rats exposed to malathion had lower plasma FSH, LH and testosterone levels than the controls (59). Investigated the effects of chlorpyrifos on male testes rat. Chlorpyrifos significantly decreased the epididymal and testicular sperm counts and serum testosterone in exposed male rats. Histopathological examination of the testes showed degener- ative changes in seminiferous tubules at various doses. Fertility test showed a negative result in 85% of the animals (60). Another study evaluated the rats exposed to dimethoate for 90 days and found a statistically significant decrease in the weight of testis. In light microscopic examinations, dose-related seminif- erous tubule degeneration was seen as sloughing, atrophy, germ cell degeneration, and arrest of spermatogenesis . Dimethoate may also cause a decrease in the sperm viability, motility, and density. Quinalphos, another commonly used OP insecticide reduced prostatic acid phosphatase activity and the content of fructose in the accessory sex glands as well as plasma levels of testosterone, FSH, and LH. Quinalphos is therefore supposed to have suppressive effects on the function of the accessory sex glands by inhibition of the release of pituitary gonadotropins (61).

2.11 Pyrethroid compound

A pyrethroid is an organic compound similar to the natural pyrethrins produced by the flowers of pyrethrums (*Chrysanthemum cineraria folium* and *C. coccineum*). Pyrethroids now constitute the majority of commercial household insecticides (62). In the concentrations used in such products, they may also have insect repellent properties and are generally harmless to human beings in low doses but can harm sensitive individuals (63). They are usually broken apart by sunlight and the atmosphere in one or two days, and do not significantly affect groundwater quality (64).

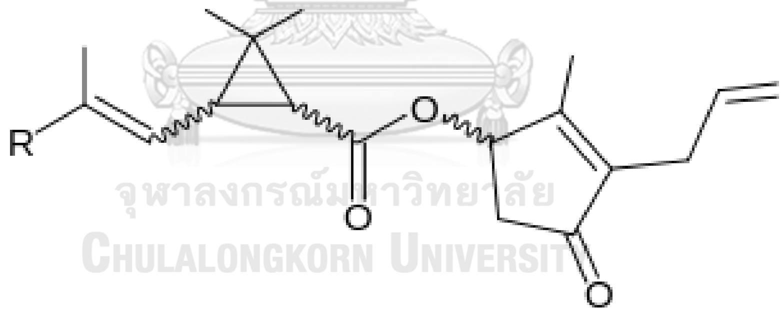

Figure 2. 8 The general structure of pyrethroid pesticide form

History of Pyrethroids were introduced in the late 1900s by a team of Rothamsted Research scientists following the elucidation of the structures of pyrethrin I and II by Hermann Staudinger and Leopold Ruzicka in the 1920s. The pyrethroids represented a major advancement in the chemistry that would synthesize the analog of the natural version found in Pyrethrum. Its insecticidal activity has relatively low mammalian toxicity and an unusually fast biodegradation. Their development coincided with the identification of problems with DDT use. Their work consisted firstly of identifying the most active components of pyrethrum, extracted from East African chrysanthemum flowers and long known to have insecticidal properties. Pyrethrum rapidly knocks down flying insects but has negligible persistence — which is good for the environment but gives poor efficacy when applied in the field. Pyrethroids are essentially chemically stabilized forms of natural pyrethrum and belong to IRAC MoA group 3 (they interfere with sodium transport in insect nerve cells).

The *1st generation pyrethroids*, developed in the 1960s, include bioallethrin, tetramethrin, resmethrin and bioresmethrin. They are more active than the natural pyrethrum but are unstable in sunlight. Activity of pyrethrum and 1st generation pyrethroids is often enhanced by addition of the synergist piperonyl butoxide (which itself has some insecticidal activity (65). With the 91/414/EEC review, many 1st generation compounds have not been included because the market is simply not big enough to warrant the costs of re-registration (rather than any special concerns about safety).By 1974, the Rothamsted team had discovered a *2nd generation* of more persistent compounds notably: permethrin, cypermethrin and deltamethrin. They are substantially more resistant to degradation by light and air, thus making them suitable for use in agriculture, but they have significantly higher mammalian toxicities. Over the subsequent decades these derivatives were followed with other proprietary compounds such as fenvalerate, lambda-cyhalothrin and beta-cyfluthrin. Most patents have now expired, making these compounds cheap and therefore popular (although permethrin and fenvalerate have not been re-registered under the 91/414/EEC process). One of the less desirable characteristics, especially of 2nd generation pyrethroids is that they can be an irritant to the skin and eyes, so special formulations such as capsule suspensions (CS) have been developed (66).

Environmental effects of pyrthroids are the fact that they are also toxic to beneficial insects such as bees and dragonflies, pyrethroids are toxic to fish and other aquatic organisms. At extremely small levels, such as 4 parts per trillion, (67) pyrethroids are lethal to mayflies, gadflies, and invertebrates that constitute the base of many aquatic and terrestriall food webs (68). Pyrethroids have been found to be unaffected by secondary treatment systems at municipal wastewater treatment facilities in California. They appear in the effluent, usually at levels lethal to invertebrates (69). Earlier studies suggested that most vertebrates have sufficient enzymes for rapid breakdown of pyrethroids, except for cats. Pyrethroids are highly toxic to cats because they do not have glucoronidase, which participates in hepatic detoxifying metabolism pathways (70). A recent study, however, suggests that developing mice exposed to deltamethrin (a pyrethroid pesticide) show neurological and behavioral changes resembling Attention-Deficit/Hyperactivity Disorder (ADHD) in humans (71).

2.11.1 Pyrethroids effect on reproductive system

Pyrethroid insecticides have been shown to affect male endocrine and reproductive function in animals; however, human data are lacking. They can decrease sperm count and motility, induce deformity in the sperm head, increase the abnormal sperm count, sperm DNA damage, and induce its aneuploidy rate, as well as affecting sex hormone levels. In a study by (72) serum reproductive hormones of 161 men recruited from an infertility clinic and their level of 3-phenoxybenzoicacid (3PBA) and cis-and trans-3-(2,2-dichlorovinyl)-2, 2-dimethylcyclopropane car- boxylic acid (cis-DCCA and trans-DCCA; pyrethroid metabolites) were determined in spot urine samples. Categories for all three metabolites and their summed values were associated with FSH. Suggestive positive relationships with LH were also found. Cis- DCCA and trans-DCCA were inversely associated with inhibinB (p for trend=0.03and 0.02,respectively). Trans-DCCA was inversely associated with testosterone and free androgen index. Permethrin binds to androgen receptors in skin cells of the human males (73). It also binds to another receptor, peripheral benzodiazepine receptor, which stimulates the production of testosterone. Cyfluthrin binds with peripheral benzodiazepine (PBZ) receptors, as well. PBZ receptors are found in the testes and are important in hormonal responsiveness (74). Six synthetic pyrethroids and naturally occurring pyrethrins can bind with androgen receptors and disrupt its normal function (73).

2.12 Pesticides effect on male reproductive system studies

The several studies related to pesticide exposure has been reported on male reproductive system. Summation of some study related to this study was reviewed and presented in Tables.

Table 2. 2 Pesticides effect on male reproductive system categorized according to pesticide's type

Reference No	Agent	Subgroup	Effect on sperm parameters
(57),(58)	Organophosphorus (OP) (animal study)		-Decreased sperm density and motility -Decreased testicular and epididymal sperm density -Decreased level of testosterone (malathion) -Decreased testis weigh -Increased abnormal sperm morphology -Decreased plasma FSH and LH -Seminiferous tubule degeneration -Decreased number of implantations and live fetuses
(73)	Pyrethroids		Interaction with human androgen receptors and SHBG -Disturbances in endocrine effects relating to androgen action (chronic contact)
(75) (76) (77)	Organochlorines	Methoxychlor 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Hexachlorocyclohexa-ne	Decreased weights of testis, epididymis, seminal vesicles and ventral prostate Increased level of hydrogen peroxide generation and lipid peroxidation in mitochondrial and micro some-rich fractions of the testis Increased abnormal sperms −Decreased testis weight Increased inflammatory cell foci in the epididymis -Decreased daily sperm production -Increased mounting and intromission latencies

Reference No	Agent	Subgroup	Effect on sperm parameters
(78)		Lindane	-Decreased serum testosterone concentration at adulthood Changed activityofsorbitoldehydrogenase,glucose-6-p-dehydrogenase,gamma-glutamyl transpeptidase, and beta-lucuronidase -Accumulation of HCH and its isomers intestes as well as sperm of treated rats
(79),(80), (81)		DDT and DDE	-Decreased serum testosterone levels, epididymal sperm count, sperm motility -Increased number of abnormal sperms Elevations in Fas, FasL and caspase-3 levels -Decreased cytoplasmic levels of NF-kappa B p65 -Changes in the localization of NF-kappa B with maximal nuclear translocation in germ cells Decreased testosterone, testicular weight, the number and the percentage of motile spermatozoa in the epididymis, and seminal vesicles weight -Increased serum LH and FSH - Inhibition of cAMP response to FSH -Decreased survival rate of sertoli cells
(66)	Carbamates	Methymol	Decreased fertility index, weight of testes and accessory male sexual glands, serum

Reference No	Agent	Subgroup	Effect on sperm parameters
(82)		Propoxur	testosterone level and sperm motility and count -Increased sperm cell abnormality -Induction of testicular lesions in seminiferous tubules and incomplete arrest of spermatogenesis
(83)		Carbofuran	Decreased sperm density per gram of cauda epididymis without affecting the reproductive performances
(84),(85)		Carbaryl	Decreased weight of epididymides, seminal vesicles, ventral prostate and coagulating glands ,sperm motility -Increased morphological abnormalities in spermatozoa Significant alterations in the activities of marker testicular enzymesSDH,G6PDH, LDH, and _-GT Distorted shape of seminiferous tubules, disturbed spermatogenesis, accumulation of cellular mass in the lumen of tubules, edema of the interstitial spaces and loss of sperms of varying degrees
(86, 87)	Dichlorophenoxyacetic acid (2,4-D)		Asthenospermia, necrospermia and teratospermia -Increased percentage of chromosome aberrations in bone-marrow and spermatocyte cells

Reference No	Agent	Subgroup	Effect on sperm parameters
(88)	Dibromochloropropane (DBCP)		Sperm count depression, elevated serum FSH but not of LH Azoospermia, decreased libido or impotence, selective atrophy of the germinal epithelium, intact sertoli cells reduced sperm counts and high levels of LH and FSH in the serum, oligospermia, germinal epithelium damage, male subfertility, spontaneous abortion, hormonal imbalances, and altered sex ratio in offspring
(89)	Benomyl		Necrosis of dividing cells, alterations in the formation of the nucleus of spermatids, occlusion of the efferent ductules, estrogenic potential sloughing of germ cells, occlusion of the efferent ducts, and blocking the passage of the sperm to the epididymis

Table 2. 3 Summary of studies investigating environmental or occupational exposures to pesticides and associations with semen quality

Reference No	Study Population	Country of study	Results
			Pyrethroids
(90)	240 men recruited from a medical university hospital	China	Significant correlation between 3-PBA levels in urine and decreased sperm concentration (β =−0.27, 95% CI:−0.41 to−0.12).
(91)	207 men recruited from an infertility clinic	USA	controlling for CDCCA. Men with the highest total DCCA urine concentrations

			had higher odds of low sperm concentrations (OR = 2.66, 95% CI: 1.07–6.92).
(92)	376 infertile men	China	Men in the highest quartile of 3-PBA urine concentration had higher odds of low sperm concentration.
			Organochlorines
(93)	311 men living in a malaria-endemic Region	South Africa	Higher p,p_-DDE concentrations were associated with decreased sperm concentration and higher p,p_-DDT and p,p_-DDE concentrations were associated with decreased motility.
(94)	207 men: 114 living below and 93 living above the Arctic Circle	Norway	Significant correlation between p,p_-DDE and higher sperm concentration (r = 0.25, p = 0.03) for men living above the Arctic Circle in Norway.
(95)	100 men recruited from an infertility clinic: 50 fertile and 50 infertile	India	Significant association between _-HCH and _-HCH and increased sperm count among infertile males. Significant associations between and _-HCH and decreased sperm counts for asthenospermic men and between _-HCH and total HCH and decreased sperm counts for oligo-asthenospermic men.
(96)	336 men recruited from infertility clinics	USA	concentration, low motility and low morphology. The risk of low motility due

			to DDT exposure was increased among men with a GSTT1 null genotype. Evidence suggests a protective effect of increasing numbers of variant alleles in the CYP1A gene against abnormal morphology.
(97)	100 men: 50 fertile men and 50 infertile Men	India	Among infertile men, -HCH, HCH, p,p_-DDE and p,p_-DDD were associated with decreased sperm counts. _-HCH was associated with decreased motility.
			Organophosphates
(98)	152 farmers: 62 with a history of exposure to pesticides	Malaysia	Men with occupational pesticide exposures had higher risks for lower semen volume, lower concentration, higher abnormal morphology and decreased sperm motility.
(80)	54 agricultural workers	Mexico	For men with the 192RR genotype of the PON1Q192R gene, there was a significant negative association between OP exposure three months before sampling and decreased sperm viability.
(99)	18 environmentally exposed men	China	Concentration was significantly lower among men with higher levels of urinary DETP. Suggestive, but not significant, association between high pesticide exposure and lower sperm concentration.

(100)	189 men: 94 cases and 95 controls	China	Men with sperm concentration and total motility below the population median had higher levels of urinary DMP compared to controls.
(63)	52 men: 17 non-occupationally exposed men, 16 agricultural workers and 19 OP sprayers	Mexico	Exposure to OPs was associated with decreased semen volume and decreased sperm count for men in the highest exposure group (OP sprayers).
(101)	62 men: 31 exposed (OP sprayers) and 31 non-exposed	Peru	Ethylated OP metabolites in urine were significantly associated with decreased semen volume.
			Other/non-specific pesticides
(102)	40 men: 20 farmworkers and 20 non-exposed men	Turkey	ABM exposed men had significantly decreased sperm motility and increased semen volume compared to men in the unexposed control group.
(103)	402 men recruited from an infertility clinic	France	Suggestive, but not significant, association between non-specific occupational pesticide exposure and altered semen (adjusted OR = 3.6, 95% CI: 0.8–15.8,adjusted p = 0.087).
(104)	87 men: 42 exposed banana plantation workers and 45 controls	Guadeloupe	No statistically significant associations between non-specific occupational pesticide exposures and semen parameters

2.13 Classification of pesticide by hazard

The human dose-effect and dose-response relationships should be known for each pesticide in order to be able to establish safety standards and to classify them according to the degree of health risk. For most pesticides, theses relationships are not known and preventive measures have therefore been developed on the basis of LD_{50} and other crude measures of the dose-response relationship in animals. WHO(1990) have grouped formulated pesticides by degree of hazard in the following table (54).

Table 2.1 Classification of pesticides according to degree of hazard to human beings (54)

WHO Recommended Classification of pesticides by hazard					
Hazard Class		*LD50 for Rats(mg/kg body weight)*			
		Oral		*Dermal*	
		Solids	*Liquids*	*Solids*	*Liquids*
IA	*Extremely Hazardous*	*5 or less*	*20 or less*	*10 or less*	*40 or less*
IB	Highly Hazardous	5 – 50	20 - 200	10 -100	40 – 400
II	Moderately Hazardous	50 -500	200 – 2000	100 – 1000	400 – 4000
III	Slightly Hazardous	Over 500	Over 2000	Over 1000	Over 4000

[a] A dosage of 5 mg/kg of body weight is equal to a few drops ingested of a splash in the eye. 5-50 mg4/kg of body weight equals up to one teaspoonful, and 50-500 mg/kg of body weight corresponds to up to two teaspoonful.

[b] The terms “solid” and “liquid” refer to the physical state of the product or formulation being classified.

2.14 Toxicity of pesticide

All pesticides must be toxic or poisonous to kill the pests they are intended to control and thus are potentially hazardous to people and animals as well as to pests. Since pesticide toxicity varies widely, it is very important for persons who use pesticides or those who regularly come in contact with pesticides to have a general knowledge of the relative toxicity of the products that are being used. Acute toxicity is usually expressed as LD 50 (Lethal Dose 50) and LC 50 (Lethal Concentration 50). This is the amount or concentration of a toxicant (the active ingredient) required to kill 50 percent of a test population of animals under a standard set of conditions. Acute toxicity values of pesticides, based guidelines for the safe use of pesticides on a single dosage, are recorded in milligrams of pesticide per kilogram of body weight of the test animal (mg/kg), or in parts per million (ppm). LD 50 and LC 50 values are useful in comparing the acute toxicity of different active ingredients as well as different formulations of the same active ingredient (54).

The lower the LD 50 or LC 50 of a pesticide product, the greater the toxicity of the material to people and animals. Pesticides with high LD 50s have the least acute toxicity to man when used according to the label directions. Pesticide products are categorized on the basis of their LD 50 or LC 50 values. Those pesticides that are classified as having high acute toxicity on the basis of either oral, dermal, or inhalation toxicity must have the signal words DANGER and POISON (in red letters) and a skull and crossbones symbol prominently displayed on the package label. Effective December 31, 1984, the Spanish equivalent for the word DANGER, PELIGRO, must also appear on the labels of highly toxic chemicals. As little as a few drops of such a material taken orally could be fatal to a 150-pound person. Acute (single dosage) oral LD 50s for pesticide products in this group range from a trace to 50 mg/kg. Pesticide products considered to have moderate acute toxicity must have the signal word WARNING (AVISO in Spanish) displayed on the product label. Acute oral LD 50s range from 50 to 500 mg/kg. From 1 teaspoon to 1 ounce of such a material could be fatal to a 150-pound person (54).

2.15 Route of pesticide exposure (105)

In a daily typical life, three primary routes of exposure relevant to humans:

1) Inhalation or breathing through the mouth and or nose
2) Through the skin or dermal exposure route- whether intact or through open cuts as well as eyes
3) Ingestion such as drinking liquids or blended foods and eating solid foods

Three other highly relevant routes of exposure to humans are worth noting:

4) Across the placenta (in blood) from a mother to the developing fetus
5) Breast milk a mother to an infant/toddler
6) Intravenously for prescription or illicit drugs

The type of exposure can vary from extreme cases of intentional pesticide poisoning (when one large dose is ingested) to occasional, low-level dosages from pesticide residues in food or water. Of particular concern in the study is the long-term, unintentional, occupational exposure to pesticides of the farming community. Most at risk are those who are frequently mixing and spraying pesticides over many years. People working in newly sprayed fields and family members in close proximity to spray equipment, contaminated clothes, and pesticide containers are also subject to some exposure. In addition, there may even be pesticide residues in rice and other food taken from the fields such as fish and frogs, and some contamination in the water supply. The lower the concentration, the less toxic it is likely to be. However, additives (the "inactive ingredients") in the formulation can affect or alter the properties of the pesticide, such as changing absorption through the skin. They may also be toxic themselves.

For dermal exposure, there are two measurements are usually made in all working environment which are associated with use of pesticides:

1. Prospective dermal exposure: The whole amount of pesticide approaching into contact with the protective clothing, work clothing and skin.
2. Definite dermal exposure: the quantity of pesticide pending into contact with the stripped skin and the fraction transferring through defending and work clothing or via closure to the underlying skin, which is therefore available for absorption (106).

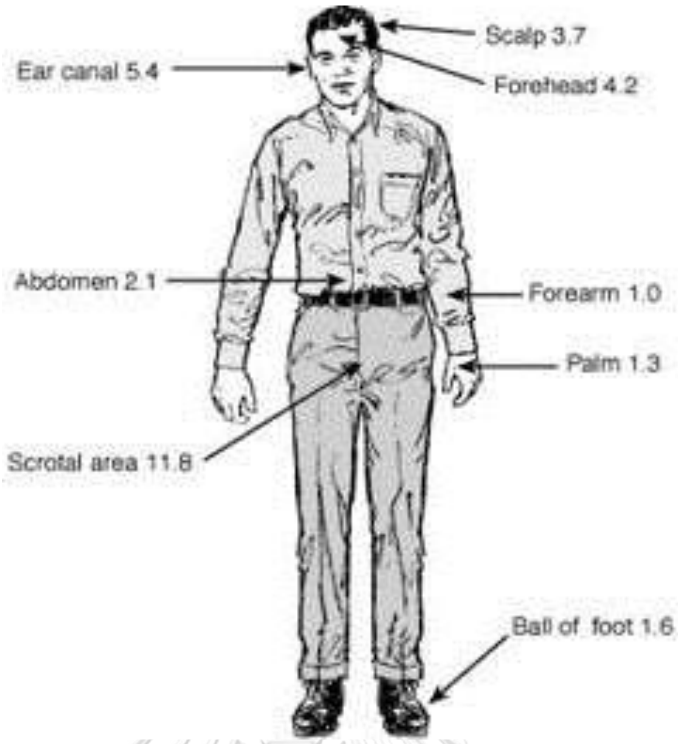

Figure 2.1 Rates of absorption through the skin are different parts of the body(31)

For inhalation exposure, long-term exposure to chemical irritants such as pesticides can cause respiratory symptoms such as cough, cold, sputum formation, wheezing, rales, tenderness, and decreased chest expansion. Incipient lung disorders can be detected by a thorough physical examination and adequate medical history. Bronchial asthma and other abnormal lung findings are two respiratory tract indicators of pesticide exposure (107).

For oral exposure, pesticides usually accidentally enter the gastrointestinal tract through the mouth. For example, a farmer who is applying pesticides and who smokes or wipes sweat off near the mouth may unknowingly ingest pesticide particles. Carbamate insecticides formulated in methyl alcohol and ingested can cause severe gastroenteritis irritation. Given in large doses to experimental animals, 2, 4-D and organochlorines are moderately irritating to the gastrointestinal lining and cause vomiting, diarrhea, and mouth ulcers. Organophosphates and copper salts also irritate the gastrointestinal tract, and irritations are manifested as intense nausea, vomiting, and diarrhea. The health indicator, chronic gastritis is clinically characterized by epigastric tenderness and pains associated with nausea and vomiting (107).

2.16 Health effects of pesticide exposure on male reproduction

Exposure to pesticides affects many body organs including reproductive system. Disorders of the reproductive system lead to infertility and therefore have been in the center of attention within the recent decades. Pesticides are one of the compounds that might reduce the semen quality in the exposed workers according to current knowledge. Although many underlying mechanisms have been proposed, the mechanism of action is not clarified yet. Majority of pesticides including pesticides including organophosphates affect the male reproductive system by mechanisms such as reduction of sperm density and motility, inhibition of spermatogenesis, reduction of testis weights, reduction of sperm counts, motility, viability and density and including sperm DNA damage, and increasing abnormal sperm morphology. Reduced weight of testes, epididymis, seminal vesicle, and ventral prostate, seminiferous tubule degeneration, change in plasma levels of testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), decrease level and activity of the antioxidant enzymes in testes, and inhibited testicular sterioidogenesis are other possible mechanisms(39).

The most important organs of the reproductive system are the external genitalia and internal organs including gonads that produce gamete cells. The whole male reproductive system is contingent on hormones which handles the activity of cells and organs. In addition, hypothalamus release gonadotropin-releasing hormone (GnHR) into the pituitary portal circulation which boosts the pituitary synthesis and discharge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The spermatogenesis lasts 72 days and then the sperms are stored in the epididymis for 2 to 4 weeks. During ejaculation, spermatozoa are mixed with a prostate’s buffered transport fliud. Seminal vesicles along the vas deferens provide fructose, the main energy resource for the system (32).

2.17 Blood Cholinesterase testing

Acetylcholine plays an important role in the functioning of the nervous system which is the cause of toxicity, resembling kinds of cholinesterase are found in the blood with the possibility to be used as indicators of exposure to OP pesticides. Op pesticides stick to both AchE that is found in the synapses and joined to red blood cells and bind to butyryl cholinesterase called likewise plasma cholinesterase (PChE) and found in plasma. It is possible for pesticides to have various affinities for AChE and PChE. Therefore, power as an inhibitor changes according to the specific pesticide. Levels of inhibition of AChE as well as PChE give not much difference information.

Effects caused by AChE inhibition may be a result of action on neurons in the central nervous system and/or the peripheral nervous system. Inhibition of acetylcholinesterase in the nervous system (both central and peripheral) is generally accepted as a key component of the mechanism of toxicity leading to adverse cholinergic effects. The inhibition of acetylcholinesterase is a key step in the mechanism of toxicity of certain organophosphorous and carbamate pesticides and, therefore, measures of cholinesterase inhibition represent a critical biochemical biomarker of potential adverse effects. Red blood cell measures of acetylcholinesterase inhibition, if reliable, generally are preferred over plasma data. The rate of turnover for red blood cells is not quick (approximately 3 months). AChE measurements also show the nature of this replacement rate that is not fast. Therefore, AChE is characteristically used as an indicator of chronic exposure. On the other hand, PChE turnover is much more rapid. PChE is a more efficient short- term indicator because of its faster response to exposure (108).

Since the red cell contains only acetyl cholinesterase, the potential for exerting effects on neural or neuro effector acetyl cholinesterase may be better reflected by changes in red blood cell acetylcholinesterase than by changes in plasma cholinesterases which contain both but plasma cholinesterase and acetyl-cholinesterase in varying ratios depending upon the species (109).

The Test-made ChE Cholinesterase Test System is on the basis of Ellman method. Acetylthiocholine (AcTC) or butyrylthiocholine (BuTC) is hydrolyzed by AChE or PChE, respectively, producing carboxylic acid and thiocholine with reaction to the Ellman reagent (DTNB, dithionitrobenzoic acid) so as to create a yellow color that is gauged spectrophotometrically at 450 nm. The rate of color information is a proportation to the amount of either AChE or PChE (110) .Cholinesterase tests can be repeated during times when organophosphate and carbamate insecticides are being used and then compared with the baseline level. The purpose of routine cholinesterase monitoring is to enable a physician to recognize the occurrence of excessive exposure to organophosphates and carbamates.

If a laboratory test shows a cholinesterase drop of 30 percent below the established baseline, the worker should be retested immediately. If a second test confirms the drop in cholinesterase, the pesticide handler or agricultural worker should be removed from further contact with organophosphate and carbamate insecticides until cholinesterase levels return to the pre-exposure baseline range. Primary care physician can help to establish the frequency of this testing program. All pesticides have the potential to be harmful to humans, animals, other living organisms, and the environment if used incorrectly. The key to reducing health hazards when using pesticides is to always limit the farmer’s exposure by wearing PPE and use a low-toxicity pesticide when available. Reading the label and practicing safe work habits will minimize hazards from the use of pesticides (111).

2.18 Risk assessment

Risk assessment is the process of identifying and evaluation adverse events that could occur is defined scenarios. It is the tool to predict the possibility of adverse effects to man and to identify the need of preventive actions. Risk assessment allows itself determining: the magnitude of the adverse effect posted by the p exposed: the characterization of risk to express of adverse effects due to actual or predicted circumstances of exposure, and the nature and severity of such effects(112).

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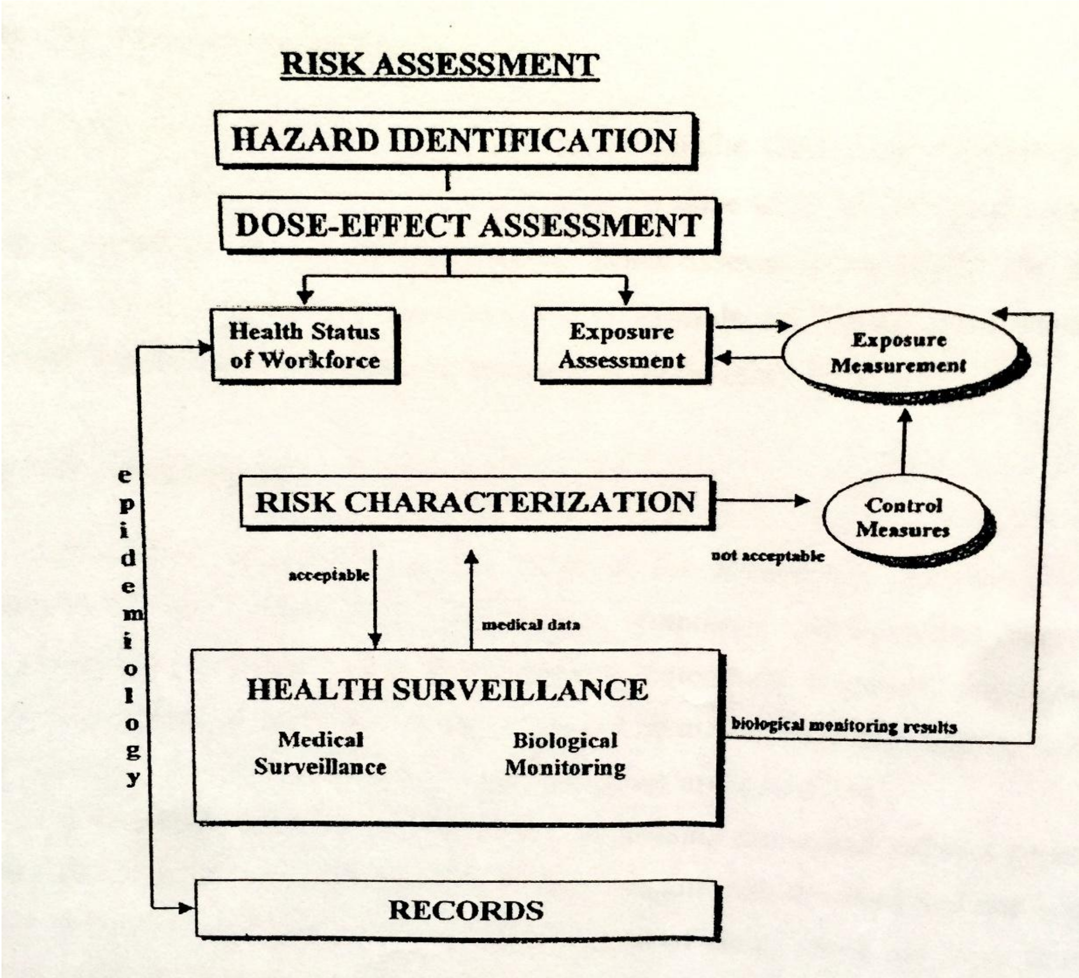

Figure 2.2 Risk assessment framework (112)

2.18.1 Hazard Identification

Risk assessment first stride that is an important process of settle on when exposure of chemical give to raising adverse effects in human health. This step used to appraise harmful of substances that standed on data collection, which includes chemical, behavior of chemical, and physical of substances, exposure route and toxicity of substances. OPs are classified as non-carcinogens substance(113).

2.18.2 Dose-response Assessment

Connection of dose and response illustrate the potential and severity of adverse health effects that are alarmed with amount and condition of exposure route. In the main, as the dose increases, the measurement of response will increase also and there may be no response at low doses. There are two steps of dose-response assessment. Firstly, all data collection or experimental data is assessed in order to find range of observed doses. Secondly, adverse health effects are estimated under the lower range of available observed data for make implications about critical regions where the dose levels begin to trigger the risk in human population. A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level that has not statistically or biologically increases. In cases that NOAEL has not shown in experiment, the term of lowest-observed-adverse-effect level (LOAEL) is used, which is the lowest testing. Reference dose (RfD) is an estimation of a daily route exposure to human population who is likely to be without a considerable risk of harmful effect during a lifetime (113).

2.18.4 Risk Characterization

Risk characterization is the final step of risk assessment. It consists of combining the information from the other steps in order to estimate the level of response for the identified health effects at the specific level of exposure to the agents of interest in the defined population. The result of risk can show as relative risk also known as attributable risk or the excess risk. Each of the risk measure adjusts the estimated probability of response in an exposed individual by the background probability of response in a different manner (114).

2.18.3 Exposure Assessment

Exposure assessment is thethe process for identifying potentially exposed populations and quantifying exposures, which includes intensity of chemicals, frequency and duration time of contact, intake rates, resulting dose, and the route of exposure (113). There are two ways that chemicals get into human body. First step is contact or exposure and Second step is cross the boundary (115). Dermal exposure is a primary route exposure in pesticides; most of farmers in the paddy field have to focuses on pesticide residues, which deposit on skin and absorb to their body.

2.19 Studies in other countries for knowledge and practice upon pesticides

The study was done in Ethiopia in which pesticide sprayers from five state-owned agricultural farms were evaluated their knowledge, attitude and practice of pesticide use on farms. To minimize risk from pesticide application, 63% was recommended avoiding applications during windy and sunny weather, 32% was suggested the provision and proper use of personal protective device (PPD), while only

3% of them felt medical check-ups and training were important, and 2% was suggested risks from spraying were best controlled by leaving their job. The hygiene and sanitation practices of the sprayers require much improvement. An attitudinal change is needed, together with the provision of better facilities and infrastructure. Pesticide safety education should be given to the sprayers. In addition, appropriate personal protective device should be used, with regular maintenance and timely replacement of worn-out parts (116).

The study done in the Gaza Strip showed that farm workers had high level of knowledge on health impact of pesticides (97.9%) and high level of knowledge on toxicity. Most farm workers were aware of the protective measures to be used during applying pesticides. Burning sensation in eyes/face was the commonest symptom (64.3%). The prevalence of self-reported toxicity symptoms was dependent on mixing and use of high concentrations of use of pesticide. The highest percentage of self-report toxicity symptom was found among farm workers who returned to spray fields within one hour of applying pesticides (117).

The study conducted in Lebanon showed that a group of agricultural workers was compared to workers of the general population and a third group of pesticide distributors. Agricultural workers were exposed to pesticides during cropping, mixing, loading, and the application (100%) but they had low pesticide knowledge than pesticide distributors and the general population workers. The preventive measures they took were low, and the lower their knowledge was, the lower were the preventive measures applied (118).

The study done in Thai farmers in Don Khasub district, Bang Phae district, Ratchaburi Province showed that the knowledge, attitudes and practices (KAP) concerning the safe use of pesticide. Research findings showed that the mean scores of KAP in the post test were significantly higher than the pretest. The results of this study provided health professionals with information to develop more effective prevention and intervention programs. To prevent illness, the most important role of health officers should be focus on education and information for individuals, families, and communities (119).

This study was shown that knowledge, attitude and practices for identifying pesticide risks by gender and to recommend more gender-sensitive programs. This study, thus, interviewed a total of 325 males and 109 females during 2005 to assess gender differences on pesticide use knowledge, attitude and practices Almost all respondents were aware of negative impacts of pesticide use on human health and environment irrespective of gender; however, females were at higher risk due to lower level of pesticide use safety and awareness. It is strongly recommended to initiate gender-sensitive educational and awareness activities, especially on pesticide use practices and safety precautions (120).

A pesticide safety knowledge test was developed to assess farmer‘s knowledge related to pesticide safety at two districts of southern Punjab Pakistan. More educated and adult respondents performed better than younger and illiterate. Similarly large land holder scored higher than small landholders, indicating their more access to information and extension (121).

The study done in an agricultural community in Palestine showed that there was significant correlation between the knowledge and safety procedure scores. Unsafe behavior were identified as the storage of pesticide products at home, the preparation of pesticides in the kitchen, inadequate disposal of empty containers, eating and drinking during pesticide application, using inadequate protective clothing. The study illustrated there was strong significant negative correlation between self-reported toxicity symptoms and scores for protective measures (66).

This study showed that use of pesticide safety practices and personal protective equipment (PPE) is greater when farmers provide decontamination supplies. Improvement of housing and workplace conditions are crucial to increase use of pesticide safety practices and PPE (122).

The study done in Oyo state, Nigeria revealed assessment of pesticide use among maize farmers. This study assessed the harmful and beneficial effects of pesticide use in maize production. It also assessed the knowledge, attitude and practice of farmers about the use of pesticides. Majority 65% found pesticide to be harmful while 39 percent found it to be beneficial. About 95 percent make use of hygienic practices while large numbers 66% neglect safety rules due to poor education and awareness. The study showed that there was evidence of excessive use of pesticide by farmers which consequently affects their health negatively (123).

The study done in Egypt showed that higher level of education and lower level of internal belief were related to better knowledge and safer use of pesticides among Egyptian farmers. The average age of farmers was 34 years and 61% of them didn't receive school education. School education was related to higher level of knowledge and behaviors. Farmers who received school education had more knowledge about the negative effects of pesticides on health and routes of contamination with pesticides (124).

This study revealed that level of Knowledge and Practice of safety pesticide use among various farm workers in agricultural field in Yerpedu, a Mandal in Chittor District of Andhra Pradesh State, India. Certain level of education and experience has contributed significant knowledge on safe use of pesticides which further has to make them to practice correct methods while applying pesticides. But no such practice has been identified which tells the need of special training to implement known safety measures rather than knowing further. The study showed that age and gender have not influenced their knowledge and practice on safe use of pesticides (125).

KAP analysis conducted at Pondicherry, India discloses that, while 70% of respondents' perceived pesticide spraying affects a person's health, only (40%) were aware that it affects the environment. Two thirds of the respondents (62%) were aware that pesticide enters the body through nose and affects lungs. Awareness on other modes of entry was less. Majority (76%) of them were aware of training programs conducted by government agriculture department on pest management. About 42% of farmers had good knowledge regarding pesticide. Between 40% and 70% of respondents was not using any protective equipment during pesticide spraying. Around 68% of farmers indiscriminately disposed empty containers while 48% buried the leftover pesticides. Significant association was observed between knowledge of the farmers and their practices related to pesticides (126).

The study done in occupational health clinic, Bahrain revealed that pesticide handlers are unaware of the pesticide exposure level. They do not read instructions on pesticide packages. The use of personal protective equipment is low. The study revealed the need for pesticide safety education and training (127).

The study done among small scale farmers in Uganda showed that farmers did not use the most hazardous pesticides (WHO class 1a and 1b). Using of WHO class II pesticides and those of lower toxicity is seen in combination with inadequate knowledge and practice among the farmers. This cause a danger of acute intoxications, chronic health problems and environmental pollution. The study showed that Integrated Pest Management (IPM) methods, use of proper hygiene and personal protective equipment when handling pesticides should be promoted (128).

2.20 Study in Myanmar for knowledge and practice upon pesticides

A study was done in pesticide formulating plant in Hmawbi Township. It was mean knowledge score of non-exposed workers with the P value of 0.0001. In exposed group regarding the knowledge, nearly all the workers have the general knowledge about pesticide. But regarding the safety measure most of the workers (72.73%) knew that watching hands after handling pesticides and only about half of the workers (48.48% and 57.88%) knew that changing clothes and bathing after handling pesticides as safety measure. Regarding the knowledge on safety equipment more than 90% of exposed workers knew mask, gloves and apron and about one third of workers knew boots and goggles and only (15.15%) few knew respirator (129).

Regarding the knowledge on poster and pictures of safety precaution in their work place very few of exposed workers knew about this. It was found that about 90% of exposed worker had good attitude towards pesticide toxicity and safety precaution. Regarding the practice on safety precaution more than 90% of exposed workers neither chewed betel nor smoked during pesticide handling and had their lunch at canteen. More than 90% washed hands, change clothes and bath after handling pesticide. One third of exposed workers used goggles and respirator during handling pesticides and 60% to 81% of workers used boots, gloves and mask during pesticide handling (129).

2.21 Related researches on Acetyl Cholinesterase (AChE) and Plasma Cholinesterase (PChE)

Sirivarasai et al. explored among 90 individuals in total exposed to OPs due to occupations and 30 controls for determining cholinesterase activity, pesticide exposure and health effects among techniques. The results proved that the correlation between occupational pesticide exposure and inhibition of cholinesterase. This study concluded that medical monitoring of cholinesterase inhibition and intervention programs with regard to safety practices during weld work are key issues with the purpose of reducing harmful health effects of pesticide to the smallest degree (130).

The study done by Kavalci et al expressed 13 patients, 7 males and 6 females, were admitted to the department of emergency because of the organophosphate poisoning who were the consequences of eating a wheat bagel in the study on evaluation of a special kind of mass poisoning, particularly by putting emphasis on the way of poisoning, the demographic aspects and clinical results of patient were analyzed. The mean age of the patients was 26±13.9 and the level of mean serum acetylcholinesterase was 2945.1±2648.9 U/L. 9 patients with supportive treatment who were given atropine and pralidoxime were hosptilized about 6.8±6.5 days. All patients recovered after the treatment with no occurrence of deaths. The failure to diagnose and treat organophosphate poisoning in a timely manner may be fatal. When cases of food poisoning are admitted to the hospital, a particular attention is need to examine if they are concerned with mass poisoning (131).

In a study conducted by Mekonnen and Ejigu's studied on cholinesterase levels in farm workers with changing exposure to chemical pesticide, plasma cholinesterase (PChE) was gauged in 82 farm workers and 47 controls workers in two Ethiopian farms. Whereas the mean values of plasma cholinesterase were in general less in workers, this difference was only meaningful in the sprayers at Birr farm. Four sprayers had cholinesterase activity less than 50% of normal. The sprayers in both farms were the groups that were affected at most, indicating that improved control on exposure to pesticide at workplace is necessary in these groups of workers (132).

Mwila et al shepherded on a research of the effect of five various pesticides (carbaryl, carbofuran, parathion, demeton-S-methyl, and aldicarb) on AChE activity was examined to determine whether the relevant combinations had an additive, synergistic, or antagonistic inhibitory effect. The related findings suggested that the mixtures involved an additive inhibitory effect on AChE activity. The data obtained from the analysis of the mixtures were used for developing and training an artificial neural network (ANN) that was then utilized with success to identify pesticides as well as their concentration in mixtures. This study is important now that it assumed mixtures of OPs and CPs whereas prior studied emphasized only either Ops and CPs. Formers studies looked into only up to three pesticides whereas the present study assessed mixtures of five pesticides concurrently. OPs constitute a group of chemical compounds used across the globe. In the United Kingdom (UK), OPs have been used in agricultural and horticultural pesticides, certain veterinary medicines especially, in human medicines (malathion only – as a treatment for head lice), as well as in different hygiene products for people in general, both for being used by professional operators (such as, for the control of cockroaches and other insect pests in public members (insecticides used in household and garden). It is concerned with the use of OPs for these objectives on which this report focused. Moreover, certain OPs have been developed as nerve agents. It is proved that acute (in other words, happening within a few days) effects on health of humans can occur following exposure to enough high levels of OPs: such effects are relevant to the acute cholinergic syndrome. Despite being rare in the UK, there have been vast numbers of causes related to severely acute OP poisoning elsewhere in the world. It is accepted that (chronic) neurotoxic effects in the long term are likely to follow occasionally those short term effects (133).

Hofmann et al pointed toward the potential risk factors for serum cholinesterase (BuChE) inhibition among agricultural pesticide operators exposed to organophosphate (OP) and N-methyl-carbamate (CB) insecticides. Use longitudinal study was conducted among 154 agricultural pesticide operators who took part in the Washington State cholinesterase monitoring program in 2006 and 2007. The analysis of BuChE inhibition with relation to reported exposures was conducted before and following adjustment for potential confounders by the use of linear regression. Additionally, ORs estimating the risk of BuChE depression ($>20\%$ from baselibe) were calculated for chosen exposures on the basis of unconditional logistic regression analyses. A whole reduction in mean BuChE activity was noticed among the participants in times of follow-up testing during the OP/CB spraying season in relation to pre-season baseline levels (mean reduction of 5.6%, $p<0.001$). Score related to estimated cumulative exposure to OP/CB insecticides in the past 30 days was an important predictor of BuChE inhibition ($\beta=1.74$, $p<0.001$). Many particular work practices as well as workplace conditions were related to greater BuChE inhibition, encompassing mixing/loading pesticides as well as cleaning spray equipment. Factors that protected against BuChE inhibition comprised full-face respirator use, wearing chemical-resistant boots as well as storing personal protective equipment in a locker at work (134).

Park et al. stated a research on two different occasions, the authors carried out a cross-sectional study of a group including 31 male farmers who used pesticides sporadically whether occupational exposures to pesticides were correlated with lessened nerve conduction studies among farmers.. Even though total median values remained within the laboratory normal limits, significant differences between the first and second tests were detected in sensory conduction velocities on the median and sural nerves, and the motor conduction velocities on the posterior tibial nerve. Duration of life days of pesticide application was negatively correlated with nerve conduction velocities at the majority of nerves following the adjustment for potential confounders. These results are likely to reflect a connection between occupational pesticide exposure and peripheral neurophysiologic abnormally that is worth additional assessment (135).

2.22 Related researches on reproductive effect in Myanmar and other countries

Thein Myint Thu et al (2010) studied in 6 villages tracts in Pyin Oo Lwin Township, Myanmar as 195 apparently healthy male agricultural workers (18-45 years) who were chronically and currently exposed to pesticides for 5 years and more were randomly selected as “test group” and 50 apparently healthy reproductive age male (18-45 years) from unexposed (control groups) and information obtained by 3 means: interview, clinical examination and laboratory investigations (seminal profile, the liver and kidney functions and the hemoglobin level) and resulted for semen analysis : sperm motility (Nil motility = 8.2% ,1-50% motility = 80%, 50-100% motility = 11.8%), sperm morphology (1.5% abnormal, 98.5% normal), sperm count (0-60 million/ml = 30.8%, >60 million/ml = 69.2%), semen volume (<2ml = 23.5%, >2ml = 76.5%) in the exposed group. In control group, sperm motility (Nil motility = nil,1-50% motility = 52%, 50-100% motility = 48%), sperm morphology (9.4% abnormal, 90.6% normal), sperm count (0-60 million/ml = 4%, >60 million/ml = 96%), semen volume (<2ml = 10%, >2ml = 90%). In view of these findings, male reproductive dysfunction seems to be associated with chronic pesticides exposure and further more elaborate and detailed study was need (6).

John D. Meeker et al studied have reported that pyrethroid insecticides affect male endocrine and reproductive function among 161 men from an infertility clinic between 2000-2003 and measured serum reproductive and thyroid hormone levels, were positively associated with FSH (all p values for trend < 0.005). Statistically significant or suggestive positive relationships with LH were also found. And also associated with testosterone and free androgen index (the ratio of testosterone to sex hormone binding globulin; p for trend = 0.09 and 0.05 respectively) and suggested for further research was need for a better understanding of the potential association between pyrethroid insecticides and male reproduction (136).

Feroz Hossain et al studied among male farmers from 3 different communities in Sabah, Malaysia at 2013 for determining the relationship between semen quality and exposure to pesticide residues. The resulted of mean values of volume, $_{p}$H, sperm concentration, motility and WBC count were significantly less in the exposed group than in compared with the non-exposed group, with p values of less than 0.05. The comparison between semen qualities such as low sperm count, motility and higher percentage of sperm abnormality of those exposed to different types of pesticides (paraquat and malathion) showed no significant differences (137).

Melissa J. Perry reviewed among 20 studies on effects of environmental and occupational pesticide exposure on human sperm. Among 20 studies ; 13 studies reported an association between exposure and semen quality; 6 studies evaluating DNA damage, of which 3 reported an association with exposure; and 6 studies assessing sperm aneuploidy or diploidy, of which 4 reported an association with exposure (138).

2.23 Related researches on Dermal Exposure and Risk Assessment in other countries

Lappharat et al. evaluated dermal exposure to chorpyrifos in 35 rice farmers along with providing a health risk assessment. Patch sample techniques was used to evaluate dermal exposure. The chlorpyrifos residue was analyzed from the gauze patches by gas chromatography (GC-FPD). The results revealed that chlorpyrifos concentrations were greater in males (526.34±478.84 mg/kg) than females (500.75±595.15 mg/kg). The hazard quotient (HQ) at the mean and 95th percentile was found to be greater than acceptable (HQ>1) (139).

Jaipieam et al. determined level of inhalation exposure to organophosphate pesticides and assessed health risk among 33 vegetable growere and 17 controls (n0n-exposed to pesticide) living in the Bang-Riend Subdistrict, Songkhla Provinces, Thailand. Personal pumps with sorbet tubes were used for air sampling during wet and dry seasons. All samples were analyzed by using gas chromatography (GC-FPD) to detect the pesticide residues, including chlorpyrifos, dicrotofos, and profenofos. The results revealed that median concentrations of three pesticides in farm areas ranged from 0.022 to 0.056 mg/m^3. In non-farm area, the samples were below the limit of detection (LOD). The concentrations of each pesticide in farm areas were significantly greater than in non-farm areas during both seasons. However, the concentrations of each pesticide between wet and dry seasons did not significantly differ. Besides, the results of risk assessment found that vegetable growers may be at risk for acute effects from exposure to chlorpyrifos and dicrotofos via inhalation route during pesticide application consisting of mixing, loading, and spraying (140)

Norkaew (2012) assessed residential exposure to pesticide residues, including OPs from agricultural and PYs from households through multiple pathways among 54 occupational households in agricultural community, Ubonratchathani Province, Thailand. All samples were analyzed by gas chromatography. The resulted showed using household pesticides for pest control in their house (73%). OP residues were detected in air samples (22.2%) and surface wipes (21.3%), wheras PY residues were detected in surface wipes, hands, foot (141).

Siriwong et al evaluated the potential health risk associated with organochlorine pesticide residues (OCPRs) contamination through freshwater organism consumption among local population in agricultural area, central Thailand. Samples of vegetables, prawn, snail, and fish were collected from canal (Khlong 7 in Pathum Thai Province). They were extracted and then analyzed by gas chromatography (GC-µECD). The findings showed low concentrations of OCPRs at levels of parts per billion (ppb). The local population could be at risk for cancer due to contaminated fish consumption with OCPRs when calculating based on a worst-case scenario (142).

Ooraikul researched risk assessment of OPPs from chili consumer at Hua Rua sub-district, UbonRachatani province, Thailand. The 110 consumers (45 males and 65 females) were interviewed. The result showed the average chili intake rate of this area was 0.018 kg/day, which was higher than the average of general Thais. Thirty-three chili samples were extracted using QuEChERS method and analyzed by GC-FPD that found chlorpyrifos (0.010 - 1.303 mg/kg) and profenofos (0.520 - 6.290 mg/kg. Both of chlorpyrifos and profenofos contaminated samples were higher than the MRLs. The ADD of chlorpyrifos from chili consumption was 1.07 x 10-4 mg/kg-day and ADD of profenofos was 8.00 x 10-4 mg/kg-day, which means profenofos exposure is higher than chlorpyrifos for chili consumers. Hazard quotient (HQ) of chlorpyrifos was lower than the acceptable level (HQ<1) and HQ of profenofos was greater than the acceptable level (HQ>1). The researcher suggested that correctly pesticide usage should be trained in Hua Rua area to minimize the risk for chili consumers (143).

Taneepanichskul studied dermal exposure in chili-growing farmer during growing season to assess risk of chlorpyrifos in chili farmers at Hua Rua sub-district, UbonRachatani province, Thailand that found chlorpyrifos residues on 35 chili-growing farmers' hands after spraying by using hand-wiping technique to collect samples. The average daily dose (ADD) of farmers was 2.51 % 10-9 mg.kg-1!day. The ADD of male farmers was higher than female farmers. Both Hazard Quotient (HQ) of male and female farmers was lower than acceptable level. The summary was not at risk with noncarcinogenic effects from dermal exposure however the researcher recommended that inhalation and oral exposure routes should be estimated risk assessment due to the farmers had mentioned on acute and back and forth or prolonged effects of OPPs after their application (67).

2.24 Study area history and health care services in Kyauk-Kan Village, Nyaung U District, Mandalay region, Myanmar

Mandalay Region is located in middle and central region of Myanmar and the population was estimated at 11.95 million in 2014 census and the second most crowded population in Myanmar located in the center of the Myanmar country. Most of the ethnic group residing in Mandalay region is Bamar. The division consists of seven districts, which are subdivided into 30 townships and 2,320 wards and village-tracts. The seven districts are: Kyaukse District, Mandalay District, Meiktila District, Myingyan District, **Nyaung-U District**, Pyinoolwin District and Yamethin District.

Under the Nyaung-U district, Nyaung-U township is the main township and population about 197,746 and Ngathayouk which area like as sub-township and population are 41,967.There are 20 villages under Nyaung-U district and Kyauk-Kan village is one of them. In Nyaung-U district, there are only 1 district hospital and 6 RHC. For health care utilization services, population about doctors are 24, nurses are 50, midwives are 42 according to 2014 census in this district. Every 20 villages do not have RHC and one RHC cover at least 3 or 5 villages together and in this Kyauk-Kan village do not have RHC properly. According to official education statistics in Mandalay region, over 1 million students were enrolled in the division's 4467 primary and secondary schools in 2005.Of the total, the vast majority, about 4000, were primary schools. Only about 13% of primary school students make it to high school. But in Nyaung-U district, most of the education status are middle school level . Kyauk Kan Village of Nyaung-U District, Mandalay Region is one of the area of Dry Zone in Myanmar. Dry Zone receives limited rains compared to country averages. This said, climate is not homogenous across the area, with conditions ranging from semi-arid (and even arid) in this areas to semi-humid in others. Dry spells during the rainy season are frequent, but their intensities vary geographically and over time. Accordingly with the climate, this area grows ground-nut as their occupation and agriculture is the primary economical source of livelihood in that area(144).

CHAPTER III
RESEARCH METHODOLOGY

3.1 Study Design

The research design of this study was a cross-sectional survey. In this study involved three phases. The first phase was cross-sectional study named as observational study, to identify the health problems related to pesticide exposure, explore knowledge and practice on safe use of pesticide among ground-nut farmers in Kyauk Kan village of Nyaung-U District, Mandalay Region, Myanmar. Data were gathered in May 14 to May 31, 2016 in the community by doing face to face interview. Total sample size in phase I were 400 (both male and female participants). For the second phase (Phase II) was also a cross-sectional study and named as laboratory study, the sample size was 100 male ground-nut farmers who were randomly selected from phase I of male participants. The phase II was to find out the effect of chemicals on male reproductive function of ground-nut farmers who were chronically exposed to different kinds of pesticides by analyzing semen, seminal hormones level and blood cholinesterase level together with physical examination. A subset of preseason baseline testing was re-estimated using a repeated measures design in (June, 2016 to October, 2016) in which large quantities of OPs are sprayed (high exposure period) growing period and non-growing period (November, 2016 to April, 2017) in which no exposure to OPs. This study design provided evaluation of within person changes in the responsive variables across time. In the third phase (Phase III), this study made the health risk assessment for exposure and risk communication by dermal exposure assess. This laboratory of phase two study started from June 8, 2016 to December 27, 2016 and phase three was done in the time of phase 2 growing period and selected 30 samples from phase 2 by simple random sampling who used chlorpyrifos pesticides for dermal exposure assessment .

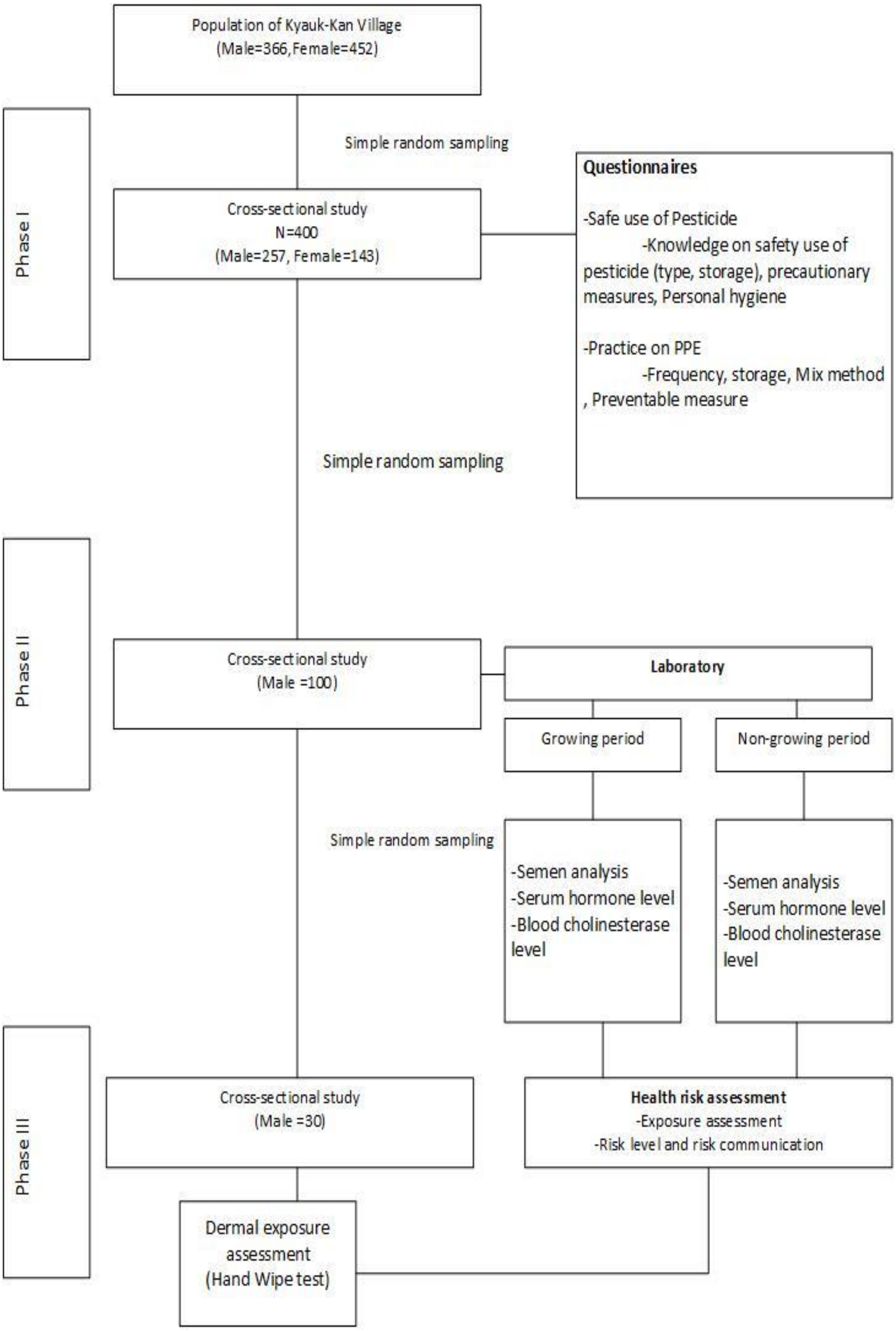

Figure 3. 1 Diagram of the Study Design

3.2 Study Area

The area of this study was purposive selection that was Kyauk Kan village of Nyaung-U District, Mandalay Region, Myanmar. This village is growing ground-nut only as their traditional occupation and one of the famous growing ground-nut zones in Myanmar for long times and had been exposed to pesticides.

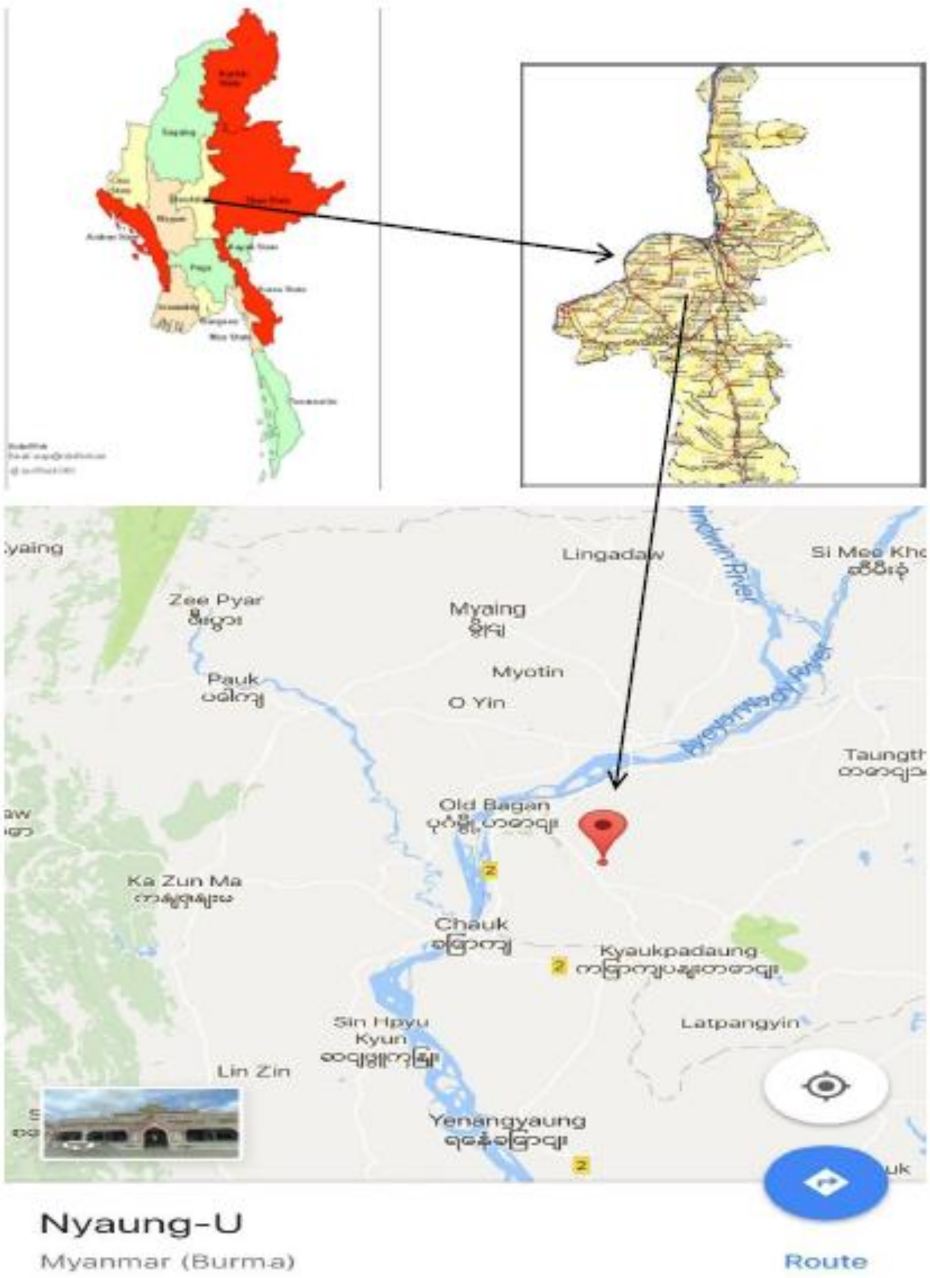

Figure 3. 2 Topography map of the study area, Kyauk-Kan Village , Nyaung-U District, Mandalay Region, Myanmar
Source: https://en.wikipedia.org/wiki/Districts_of_Myanmar (145)

3.3 Sample Size and Sampling Techniques

For phase I, Observational study,

The following formula was used for sample size determination.

$n = Z^2pq/d^2$ (Lwanga, S.K and Lemeshaw. S,1991) (146)

n = Minimum required sample size

Z = Reliability coefficient ($Z\alpha = 1.96$ for 95% confidence level)

p = The proportion of farm workers who have satisfactory knowledge on pesticide (p= 0.5, Khin Maung Nyunt, 2015) (28)

$q = 1 – p$

d = precision error

n = (1.96)2 x 0.5x0.5 / (0.05)2 = 384

Minimum requirement sample was 384 and non-respond rate was 10 percent. Therefore, at least 400 total sample size were required for phase 1 observational study.

Nyaung-U District was purposely selected. The reasons for choosing were: (a) the use of pesticides among farm workers in this township is said to be high because nearly all the farm workers use the agricultural pesticide for prevention and control of pest and (b) there has been no other study related to pesticide in this township yet. There are six Rural Health Center (RHCs) in this township. Among them, one rural health center will be selected. Then, one village from these RHC was selected by simple random sampling and Kyauk_Kan village was chosen. Agricultural workers in that village was selected by lottery method and each agricultural worker were taken as study unit. Each agricultural worker were interviewed face to face by using semi-structured questionnaires. Total number of households in this village is 182 and total number of 818 peoples is staying. Between them, male is 366 and female are 452: working age group are about 642 and non-working age group are 176. According to the data collected from township office, Ministry of Agriculture and Irrigation, it is found out that, the total farming area of these villages is 6,000 acres. The number of farm workers is 636. It means that each farm worker is working 9.43 acres (1:9.43).

For phase 2, Laboratory study,

The following formula was used for sample size determination.

$n = Z^2pq/d^2$ (Lwanga, S.K and Lemeshaw. S,1991) (146)

n = Minimum required sample size

Z = Reliability coefficient (If $Z\alpha = 1.96$ for 95% confidence level)

p = The approximate proportion of farm workers who had normal blood cholinesterase level.

(p= 0.8, Khin Maung Nyunt, 2015) (28)

$q = 1 - p$

d = precision error

n = (1.96)2 x 0.8x0.2 / (0.08)2 = 96

Therefore, minimum requirement sample was 96 and non-respond rate was 10 percent. Therefore, at least 100 total sample size were required for phase 2, laboratory study.

Generally, the longer or more often a person was exposed to a given amount of a pesticide, the greater the chance of harm. Therefore, among the 400 farm workers, 100 farm male workers who had five years above than work durations was selected for testing semen analysis and blood hormones level to find out how much pesticide expose upon male reproductive system and also finding testing blood cholinesterase level for determining the acute or chronic pesticide exposure effect and skin exposure test by using gauze patch samples method in only 30 randomized samples at phase III. This test had done for twice in growing season and non- growing season period but for pahse III, only done in growing period.

For health risk assessment, to find out the exposure, risk characterization and risk communication and physical examination was taken.

3.4 Study Population and Sample Group

Ground-Nut farmers in Kyauk-Kan village, Nyaung-U District, Mandalay Region, Myanmar, who were normally apply pesticides to the filed, will be recruited as respondents for this study. All participants were selected following to the eligible criteria:

For phase I, Observational study,

The first part was phase I named as observational study, identified the health problems related to pesticide exposure, explored knowledge and practice on safe use of pesticide among ground-nut farmers among both male and female between May 14 to 31, 2016 in the community by doing face to face interview. They were chosen through the following criteria.

Inclusion criteria	Exclusion criteria
Living in the study area more than 5 years	Hired for apply pesticides from another village
Their houses and farms are located at Kyauk-Kan Village, Nyaung-U District, Mandalay Region, Myanmar	Unwilling to answer the interview
Farmers age between 18-49 years old (Reproductive Age) (147)	
Daily work in the farm	
The participants who mix, load and spray pesticides Both male and female	

For phase 2 and phase 3, Laboratory study,

For the second and third part was also a cross-sectional study and named as laboratory study, found out the effect of chemicals on male reproductive function of ground-nut farmers who were chronically exposed to several kinds of pesticides (Organophosphate group and Pyrethroid group) by using biomarkers (semen analysis, serum hormone level and blood cholinesterase level) in two periods: 1st time growing period June 8 to 18, 2016 And 2nd time non-growing period at December 17to 27, 2016. For third phase, hand wipe test was done only in second phase growing period. Because in growing period, the participants exposed to pesticides and can be differentiated that how much pesticides exposed to the farmers by skin exposure and can be assessed for health risk. In non-growing period, the farmers have not been exposed with pesticides and just for cropping period. So it could not find as dermal exposure assessment for hand wipe test. They were chosen through the following criteria.

Inclusion criteria	Exclusion criteria
Living in the study area more than 5 years	Hired for apply pesticides from another village
Their houses and farms are located at Kyauk-Kan Village, Nyaung-U District, Mandalay Region, Myanmar	Prior vasectomy or current use of exogenous hormones
Farmers age between 18-49 years old (Reproductive Age) (147) and sampling from observational study	Unwilling to give sperm or blood samples
Daily work in the farm	
The participants who mix, load and spray pesticides Only male farmers For Hand Wipe test, 30 randomized male who use the Chlorpyrifos pesticide	

3.5 Project Procedures and Measurement Tools

Phase I Observational Study

Active contact with the community leaders, township leaders, health committee and other key persons carried out in the community. The rapport built up with the people in the community and research team. Population of ground-nut farmers updated and acquired from the selected village.

Training to facilitators

Objectives

- To train the facilitators for **good interpersonal communication and facilitating skill** which in turns building up the self-efficacy.
- To produce the facilitators who can **be able to organize and make an effective community meeting**.
- To produce the facilitators who can **be able to make an efficient training of township health volunteers**.

Three facilitators who have a master of Public Health degree and Paramedical Science degree were identified. The training of the facilitators were conducted by the researcher. The facilitators participate in training and they have mastered lesson content and delivery strategies prior to study implementation. The activities of facilitators were monitored by the researcher.

3.5.1 Pre Test

Before the main data collection, semi-structured interview questionnaire were prepared in English language and translated into Myanmar language. Then training was given to three persons, two of them who are finished in Master of Public Health Degree and one has Paramedical Science Degree Holders as well as medical doctors to perform as interviewers. In this training, the objective of this study was explained by the investigator and interviewing approaches was rehearsed with each other. From this training, they came to understand and became familiar with the procedure in data collection. Then, pre-test was done with 30 farm workers in Kong Tan Gyi village, Nyaung-U Township which is 20 miles away from the study area by the investigator and three trained persons. A Cronbach's Alpha coefficient was used to test the reliability of the questionnaires and the reliability value was 0.7. For Validity test, a set of questionnaires were checked and verified by researcher supervisors and concerned teachers.

After the pre-test, the questionnaire was discussed among interviewers and the questions that made difficult to ask or to answer were noted down and modified accordingly.

3.5.2 Questionnaire

For questionnaire interview, which contains of three parts as follows:

Part 1: the general information and personal history of the rice farmers who always apply and contact with pesticides, will be asked, namely, ages (years), gender, body weight (kilograms), height (centimeters), education level, pesticide application practices, and duration of rice farming.

Part 2 : Explore knowledge and practice on safe use of pesticide

The questionnaire was developed based on literature review and preliminary survey among ground-nut farmers in another community. The information in this part was based on safe use of pesticide utilization by using questionnaires on knowledge and practice on pesticide among ground-nut farmers.

(1) Knowledege regarding exposure to pesticide residues and protection

It was contained 14 questions. Each item was scored 1 for the right answer, and 0 for the wrong or unsure answer. Question number 1 to 7 was choosed only 1 answer for each questionnaire but question number 8 to 14, within in each question, can choose more than one answer depended on participants choiced (shown the score in Appendix D).The overall knowledge score were assessed using the sum of each outcome and the scores were classified into 3 levels based on Bloom's cut off point, consisting of low level (<60%), moderate level (60-79%) and good level (≥80%). (148).

(2) Practice regarding exposure to pesticide residues and protection

It was contained It was contained 19 questions. Each item was scored 1 for the right answer, and 0 for the wrong or unsure answer. Question number 1 to 4 was choosed only 1 answer for each questionnaire but question number 5 to 19, within in each question, can choose more than one answer depended on participants choiced (shown the score in Appendix D).The overall practice score were assessed using the sum of each outcome and the scores were classified into 3 levelsas good practice (≥ 80%), fair practice (60%-79%) and poor practice (less than 60%) and the scores were classified into 3 levels based on Bloom's cut off point (148) (Appendix D).

Part 3: Favorable Environment

The questions was asked on available, accessable , affordable upon PPE and getting information about PPE.

Main data collection had done in first week of May 14, 2016 because growing period starts growing ground-nut among farm workers from the selected village. Before the interview, the lists of farm workers who met inclusion criteria from the selected farms were taken from the respective head of village. A random selection of participants from each female and male list, made till the required sample sizes were obtained for sample size 400 by using lottery method. Face-to-face interview was conducted in the field and at home by the three trained interviewers. Data collection was done in the afternoon and in the evening to suit the farm workers. Privacy was ensured during the interview. When a selected interviewee were not presented during home visit or in the farm, a re-visit was made after inquiring his or her available time. If the interviewee still absent on the second visit, he or she was excluded.

At the end of each day, data was collected by three trained interviewers were checked by the principal investigator. The outcomes of the interview was discussed between the investigator and the three interviewers for clarification.

Face-to-face interview obtained only reported knowledge and practices. Thus, non-participant observation was also used in order to see their actual practice on utilization of agricultural pesticides and to confirm the reported practices. Observation was done by the investigator himself and three trained interviewer using a check-list (see Appendix B) after 2 weeks after completed face to face interview to 400 samples. Check list contained storage of mixing, spraying, using PPE and disposal of pesticide.

This decision was made also in consideration of time and financial limitations. During the observation evidence was made by taking photos. For ethical consideration, oral permission for taking photos and using them in the thesis were requested from the respondents.

Phase II Laboratory Study

Laboratory study was done in two periods: growing and non-growing period. In the study area, ground-nut growing need at least 6 months duration and probably, the ground-nut farmers starts growing in June to November. Among these duration, the highest using pesticides periods was in July to September. According to their growing habits, the laboratory study was done among 100 male ground-nut farmers who were selected from the observation study by inclusion criteria. By this way, first survey of growing period was done in June 8, 2016 and non- growing period was done in December 17, 2016. The researcher and two trained public health doctors and one laboratory technician were done clinical examination and collection sperm and blood hormones , blood cholinesterase test to distinguish organophosphate poisoning effect and exposure effect from skin by doing path samples form the male participants according to the guideline of world health organization (WHO) procedures and guideline in both growing period and non-growing period. But for testing semen analysis, the laboratory technician who had the bachelor degree in respective fields was searched the semen analysis under the microscopic within 2 hours after getting the sperm from the participants and searching about the changing of sperm characteristic and for serum hormone level, firstly blood samples for serum hormone analysis from male ground-nut farmers was collected in a 10 ml plastic syringe, and keep at room temperature and blood samples were centrifuged until serum separated by the researcher and laboratory technician. After centrifugation, the serum was transferred to a new tube, kept at-20˙C, and assayed within 4 weeks and all the blood samples was measured in the standard private laboratory (S.M.L Advanced Medical and Diagnostic Center in Yangon Region, Myanmar) analyzing residue of pesticide by the electro chemiluminescence immunoassay “ECCLIA”. The other procedures were done as the following procedures by the researcher and two trained public health doctors and one laboratory technician.

3.5.3 Measurement of height

(1) Setting up stadiometer at the examination site

The heights of male farmers were measured by stadiometer. A carpenter's level will be used to check the vertical placement of the rule. The floor surface next to the height rule must be hard. If no such floor was available, a hard wooden platform placed under the base of the height rule. Using the carpenter's level, the surface on which the height rule rests were checked to be horizontal.

(2) Calibration of height rule

At the beginning and end of each examination day, the height rule was checked with standardized rods and corrected if the error was greater than 2mm. The results of the checking and recalibrations were recorded in the log book.

(3) Normal Height measurement procedure

1. Participants were asked to remove their shoes, heavy outer garments, and hair ornaments.
2. The participant were asked to stand with his/her back to the height rule. The back of the head, back, buttocks, calves and heels have being will touch the upright, feet together. The participant was asked to look straight.
3. The head piece of the stadiometer or the sliding part of the measuring rod was lowered so that the hair was pressed flat.
4. Height recorded the resolution of the height rule (i.e. nearest millimeter / half a centimeter). If participant was taller than the measurer, the measurer stood on a platform so that he / she could properly read the height rule.

3.5.4 Measurement of weight

(1) Setting up scale at the examination site

The scale was placed on hard-floor surface. A carpenter's level was used to verify that the surface on which the scale placed horizontal.

(2) Calibration of scale

Calibration was done at the beginning and end of each examining day. The scale was balanced with both sliding weights at zero and the balance bar aligned. The scale was checked using the standardized weights and calibration was corrected if the error was greater than 0.2 kg. The results of the checking and the recalibration were recorded in the log book.

(3) Weighing procedure

1. Participants were asked to remove their heavy outer garments (jackets, coats, trousers, etc.) and shoes.

2. The participants were asked to stand in the center of the platform, weight distributed evenly to both feet because standing off-centre may affect measurement.

3 .The weights were recorded to the resolution of the scale (the nearest 0.1kg or 0.2 kg).

3.5.5 Semen collection and seminal fluid analysis

This laboratory study was done by the researcher and two laboratory technicians who got the degree from nursing institute in the study area at both growing and non-growing periods.

For the participants security and shy, prepared for one private room to collect the sperm and all of the responsibility was given by the primary investigator. The participants should avoid from any sexual activity (including masturbation) for at least 2 days and no more than 10 days. Longer or shorter periods of abstinence may result in a lower sperm count or decreased sperm motility. A semen sample was collected by masturbation into a sterile wide-mouth glass container after a recommended period of 2-3 days sexual abstinence from male ground-nut farmers. Do not use any lubricant, including saliva, when collecting semen. The specimen should be collected in a container provided by the researcher. Be sure hands and penis are cleaned prior to collection. Avoid touching the inside of the cup. If any semen is spilled, DO NOT attempt to transfer it to the cup. Inform the lab personnel about the spill. If the specimen was obtained outside of the collection room, bring the specimen to the laboratory within one hour after ejaculation. Do not expose the specimen to extremes of temperature. Place specimen container upright in a plastic bag, with the lid securely tightened, and keep specimen close to body temperature by transporting close to the body. The specimen should not be placed in a purse, pocket, or briefcase. Sperm do not have a long life outside of the body and at different temperatures. Delays in delivering semen and exposure to various temperatures would be showed the results in lower overall motile sperm count and poor semen cryopreservation. Unacceptable specimen were defined as containers is cracked or broken or leaking. It was the adequate for the accurate assessment of semen quality. Information on date, time and spillage recorded and coded on each sample. The samples were kept in an incubator at 37˙C until liquefaction which took about 20-30 min and subsequently examined. All semen samples were processed and analyzed by qualified personnel based on WHO guidelines (149, 150). All samples were examined twice for the authenticity. Volume, $_{p}H$, sperm concentration, motility, morphology and WBC count were examined by the investigator to reach the standard quality control at the study area and recorded. If any discrepancy and error noticed, the semen analysis including the count was repeated for a third time to assure the accuracy.

3.5.5.1 Macroscopic examination

Measure the volume in a small graduated cylinder: the amount varies from only a few drops up to 10ml. The normal volume is 4-5ml. pH is usually noted through it is of little significance and normal range about 7.2 to 7.8. Freshly ejaculated semen should be completely liquefied within 30 minutes was called as viscosity (149),(150).

3.5.5.2 Microscopic examination

According to WHO criteria (149, 150), normal anatomical form of spermatozoa are length of 50-70 µm with large oval head shape, with a small neck and a long slender tail; the total length of the tail takes up about 90% and the head is 3-6 µm ^{x} 2-3 µm. The abnormalities of morphology to be looked for include: abnormally shaped heads, abnormally sized heads, double heads, coiled tails, absent, bifurcated or swollen necks and double, rudimentary or absent tails. To check the mortality, place a drop of semen on a slide, cover the drop with a coverslip and rim the edge with petroleum jelly to prevent evaporation. Examine under the ^{x}40 objective of the microscope. Normally about 80% of the spermatozoa are actively motile and 20% are sluggish or not moving at all. Decreased sperm motility may be a factor in infertility. For sperm count, 1) after liquefaction has taken place, gently shake the specimen to mix, 2) using a sahli pipette, draw semen to the 0.5 µl mark; then draw in the semen diluting fluid to the 11 µl mark and place the pipette on a rotator to mix the contents, 3) load an improved Neubauer counting chamber, allow the sperm to settle and then count in the four corner squares, as for a leukocyte count according to formula. The normal sperm count is between 60 million and 150 million per ml. Patients with sperm counts below 60 million per ml definitely have low counts, though they may still be fertile (149, 150).

3.5.6 Reproductive hormones analysis

Venous blood samples were obtained after a 12-hour overnight fast. Blood was centrifuged at 3000 rpm, and serum was collected after centrifugation and the serum were transferred to a new tube, kept at-20˙C, and assayed within 4 weeks. Serum testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured in the standard private laboratory (S.M.L Advanced Medical and Diagnostic Center in Yangon Region, Myanmar) analyzing residue of pesticide. by the electrochemiluminescence immunoassay "ECCLIA" is intended for use Elecsys and cobas e immunoassay analyzers.All samples were run in the same assay period.

Blood samples from male ground-nut farmers were collected in a 10 ml plastic syringe, and kept at room temperature and blood samples were centrifuged until serum was separate by the researcher and two laboratory technicians who got the degree from nursing institute in the study area in both growing and non-growing periods (32). Before being enroll in the study, male farmers were informed of the purpose of the study, procedures, benefits and possible risks will involve. The male farmers were told that all information as well as biological samples and data was obtained for the study was remained confidential. Written consent to participant was voluntarily obtained from all of the male farmers in this study prior to their participation. The normal value of the reproductive hormones in the ground-nut farmers were as follows:

FSH (Follicle-stimulating hormone)	-	1.5 - 12.4 mIU/ml
LH (Luteinizing hormone)	-	1.7-8.6 mIU/ml
Testosterone	-	0.2-1.4 ng/ml (151)

Figure 3. 3 Elecsys and cobas e analyzers for reproductive hormone

3.5.7 Blood Cholinesterase testing

Blood cholinesterase was used as biomarker of pesticide exposure. Specimens was collected from male ground-nut farmers by finger-prick technique (require only 10 µl of blood) after the end of their shift. It was analyzed by Test-mate ChE Kit (Model 400) that was manufactured by EQM Research, Inc because most organophosphate and carbamate chemical class can affect the blood enzymes acetylcholinesterase (AChE), and plasma cholinesterase (PChE). The entire assay was completed in less than 4 minutes. These two tests have different meanings: Plasma cholinesterase (PChE) that is found primarily in the liver is used to account for acute effect, while Acetyl cholinesterase (AChE) that is found in nerve tissue and red blood cells can account for chronic effects due to longer half-life (110). The kit is very light and portable and also easy to use tool for cholinesterase activity testing in clinic laboratory, or field research setting (152). Cholinesterase levels vary with an individual, between individuals, between test laboratories, and between test methods. Results obtained by one test might be not appropriate to compare with results obtained by another. People who exposed to cholinesterase-affected pesticides can develop lowered cholinesterase levels.

3.5.8 Exposure assessment by hand wipe sampling

Exposure assessment was the quantitative and qualitative estimate of chemicals contact, which includes intensity of chemicals, frequency and duration time of contact, intake rates, resulting dose, and the route of exposure (153). There were two ways that chemicals get into human body. First step was contact or exposure and Second step is cross the boundary. And there were two route for cross the boundary that were intake and uptake. Intake means chemical get into body through mouth or nose but if chemical cross from outside to inside body by tissue or skin absorbing, it called uptake (154).

The direct assessment of dermal exposure is accomplished by measuring the concentration or amount of the contaminant in contact with the skin over a period of time. Dermal exposure was a primary route exposure in pesticides; most of farmers in the paddy field have to focuses on pesticide residues, which deposit on skin and absorb to their body. The methods developed for such purposes have evolved from industrial hygiene practices and, generally, entail either the removal of accumulated residues from the skin or collection of the material as contact occurs. Removal techniques aim to sample the mass of material remaining on a worker’s skin at a particular point in time. Wipe sampling can show a high degree of variability in recovery efficiency and are also of limited use when the substance under study is either highly volatile or likely to be rapidly absorbed by the skin. The removal methods include uncertainties in the removal efficiency and require that the duration of contact be evaluated through independent means. Uncertainty is introduced by the collection methods through the use of materials that usually do not mimic the adherence characteristics of the skin accurately. (155).

In this study, removal procedure was done by wipe method. Solvent impregnated materials can be used to wipe the skin and remove the residues. The wipe material is then analyzed for the contaminants of concern. Washing and wiping were method that can remove chemical deposit and concentration of chemicals can be measured. Collection procedures were done by patch method. Patches made of various materials can be placed on the body to collect contaminants of interest as contact occurs. The patches are designed to have adherence characteristics similar to skin. The method requires some fairly extensive assumptions, and in the occupational setting, it has proven to be useful for screening purposes but is limited as a quantitative method. A modified version of Lappharat et al studied (139) and P. Ong-artborirak studied (156), was used to assess exposure to pesticide residues (PRs) from patch samples.

In this study, dermal exposure assessments were done by chemical removal techniques. A moistened gauze pad (4” x 4”) with 6ml of 40% propranolol in accordance with WHO sampling protocol, was used to wipe the hands of participants at the end of their work in order to measure chemical deposits and concentration of chemicals. These patches were carefully removed, wrapped in aluminum foil, and placed in a zip-lock plastic bag. All samples were stored in dry ice, transferred to the laboratory, and refrigerated at -20˙C until extraction and analysis by gas chromatography (157). Analyses was completed within seven days of the sample collection.

3.5.8.1 Hand wipe sample collection

The patch samples was conducted only in growing period and the sample were randomly selected 30 participants of ground-nut farmers among the total number of 100 participants who used chlorpyrifos (organophosphate pesticide) based on the application schedule of the ground-nut farmers, samples were taken after the heaviest application period. Chlorpyrifos residue can be detected on skin, in blood and through metabolites in urine and it has many adverse human health effects (158) and in this area, chlorpyrifos is commonly used by ground-nut farmers to protect their crops from various pets. Although there have been several pesticide monitoring studies among farmers in Myanmar, but there was no study for assessment on human risk from dermal exposure among ground-nut farmers at Myanmar. According to limitation of time and budget, only 30 samples were randomly selected and done dermal exposure assessment for this study.

Wipe samples were foiled and kept in Ziploc plastic bag, and frozen in an ice box. All samples were transported to the laboratory and stored in refrigerator at -20˙C before analysis. The analysis of wipe samples was performed at the Laboratory of the College of Public Health Sciences, Chulalongkorn University.

3.5.8.2 Hand wipe sample analysis

Firstly, standard solution preparation was carried out. A total of 10 organophosphate pesticide standards (chlorpyriofos) was selected based on the results report which pointed out the presence of pesticide residues. Stock standard solutions at concentration of 10 mg in 10 ml to get 1000 pm were prepared in hexane, acetone, and acetonitrile. In order to achieve pesticide mixture solutions at concentration level of 0.1, 0.5, 1, 5, 10, 50, 100 ppm in Acetonitrile (MeCN), calibration solutions has been obtained.

Secondly, sample preparation was implemented. Samples would be extracted by liquid-liquid extraction method. First, the patch samples were placed in 250 milliliters flask and added 40 milliliters of acetonitrile (HPLC grade) in an Erlenmeyer flask. After that they were agitated on a mechanical shaker at a high speed for 10 minutes at room temperature. Then, the gauze patch was squeezed to make the water as much as empty into the beaker and take the water out from the gauze by using the pipette or syringe until it got dry. Then the solution in beaker to be evaporated by Nitrogen gas (dispersed the solution by N_2 gas) and the water in the bath set at 40˙C until the solution remained almost dried. Then adjust the volume to be 1ml by filling the acetonitrile and 1% acetic acid into the beaker. After that 1ml of solution was transferred to Eppendorf tube and added up with $MESO_4$ 150 mg and PSA 25mg. After that it would be shaken for about 1 min and centrifuged 2 min at 6,000 rpm. Finally, the solvent was used to inject to Gas Chromatography with Flame Photometric Detector (GC-FPD) (159). A schematic of the extraction procedure was shown in Figure 3.3.

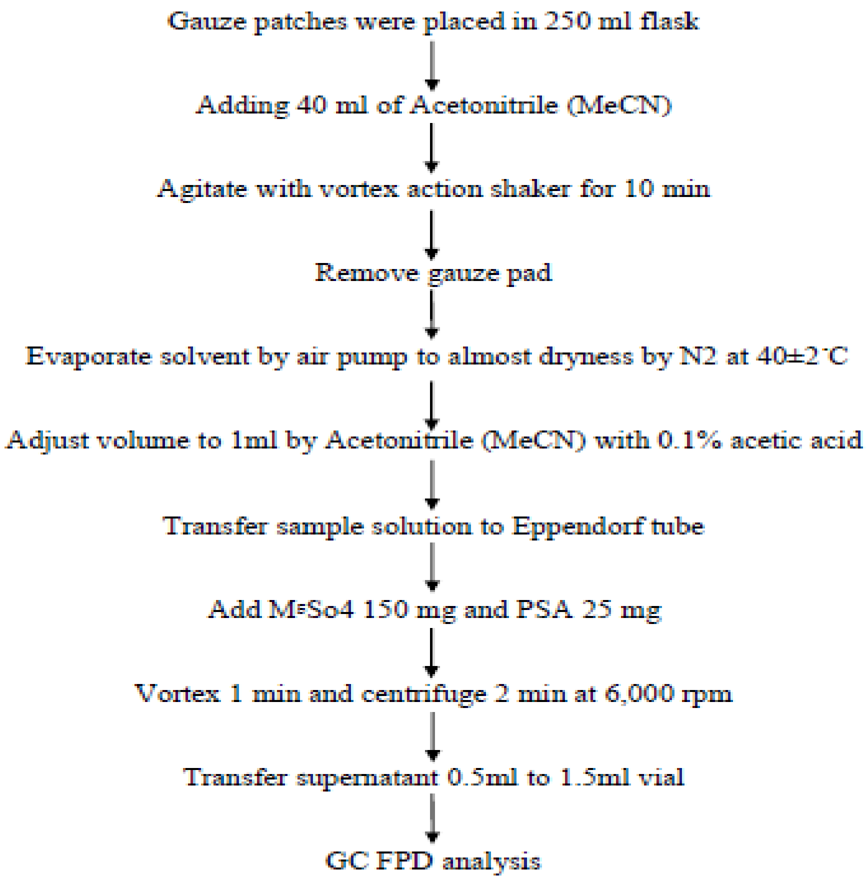

Figure 3. 4 Flow chart of the liquid-liquid extraction method(159)

Thirdly, gas chromatography analysis was prepared. In this research, Agilent 7890 equipment with Flame Photometric Detector (FPD) was used for quantification. Substances were separated by DB-5 (30.0 meters length, 0.250 millimeters diameter, 0.25 micrometers film thickness) coated with 5% Phenyl Methyl Siloxan. Samples quantification was calculated using multiple external standards. The optimum condition could be provided as shown in Table 3.1.

Table 3. 1 Analysis of organophosphate GC-FPD condition

Capillary Column	DB-5 (30.0 m length, 0.250mm diameter, 0.25 µm film thickness) coated with 5% Phenyl Methyl Siloxan
Carrier Gas	Nitrogen at 2ml/min flow rate
Make up gas	Nitrogen at 45ml/min flow rate
Detector gas	Air at 100ml/min flow rate Hydrogen at 75ml/min flow rate

Type of Injection	Spiltless			
Injection volume	1 μL			
Injector Temperature	230 ˙C			
Detector	Flame Photometric Detector (FPD)			
Detector Temperature	250 ˙C			
Oven Ramp Flow rate	**Flow rate ºC/min**	**Next ºC**	**Hold (min)**	**Run Time (min)**
Initial		100	0.00	0.00
Ramp 1	12.00	220	0.00	12.00
Ramp 2	20.00	260	1.00	15.00
Ramp 3	20.00	280	5.00	21.00

Fourth, method validation was done. Correlation (R^2) obtained from plotting calibration curve of investigated pesticide at 7 concentrations with analysis of 7 replicates, ranged was 0.99994. For the chlorpyrifos standard the calibration standard was run every 7 samples in which every measurement was performed in the range of linearity.

The limit of detection (LOD) of an individual analytical procedure is the lowest amount of analytic in a sample which can be detected, but not necessarily quantitated as an extract value. LOD is the point at which a measured value is larger than the uncertainty associated with. It is the lowest concentration of analyze is a sample that can be detected, but not necessarily quantified. In chromatography, the detection limit is the injected amount of the results in a peak with a height at least two or three as high as the baseline noise level. In this study, signal noise value is 4.0 and LOD value for this study was 0.01 µg/ml. Limit of Quantitation (LOQ) of an individual procedure as the lowest amount to analyze in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in a sample matrices and is used particularly for the determination of impurities or degradation products and can be qualified with acceptable accuracy and precision (160). If the required precision of the method at the limit of quantitation has been specified, 5 or 6 samples with decreasing amounts to analyze are injected six times. Laboratory validation was referred to the Scientific

Association Dedicated to Excellence in Analytical Method (AOAC) (160), all quality control values presented this qualitative study was in the recommended standard level. In this study, Average recoveries of chlopyrifos by analysis in this study were 7 replicates for 3 times. The range from the known LOD is determined above to 20 times the LOD. A typical signal to noise ratio was 10:1. In this study, LOQ value was 0.02 µg/ml and LOQ was greater than twice the LOD. Validation data was presented essentially as quantitative recovery in the range of 80-120% and validation data was presented essentially quantitative recoveries were presented at the level of 1 µg in the range of 101 – 113 %. The percentage of recovery value (mean) was 107.26 % and the value was between 80-120%. Relative Standard Deviation (RSD) or coefficient of variation (CV) was used to estimate the precision for multiple samples. The precision acceptance criterion depends on the type of analysis. The precision in environmental analysis depended on the sample matrix, the concentration of analyze and the analysis technique. It was measured the variation between 2% and less than 20% and the percentage of relative standard deviation value as 3.82% and within the standard (161). Retention time of chlorpyrifos was 12.205 min. The method of accuracy was calculated by percent of recovery from analysis of reference materials, or laboratory control samples.

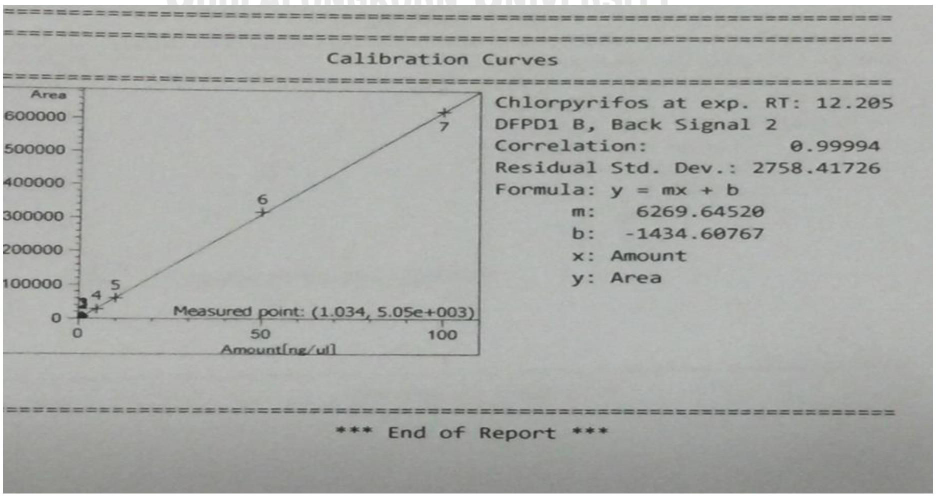

Figure 3. 5 Chlorpyrifos calibration curve

Table 3. 2 Quality Control (7 replications)

Pesticide	Calibration Curve (R^2)	LOD (μg/ml)	LOQ (μg/ml)	% Recovery	%RSD
Chlorpyrifos	0.99994	0.01	0.02	101-113	3.82

3.6 Quality Control

In terms of inter and intra observer variation was controlled by using the standard laboratory at the College of Public Health Sciences, Chulalongkorn University for analyzing residue of pesticide. For seminal analysis, all samples were prepared by the investigator to reach the standard quality control at laboratory. The SML laboratory staffs in Myanmar were assessed the analytical chemical technique to document method validation that AOAC Peer Verified Methods Program (1993) recommended (160).

3.7 Data collection

Data collection was done by researcher and well-trained researcher assistants. The process of data collection was introduced and trained to all assistants before the data collection period by group and personal training. Some sample collections, such as dermal wipes and patch sample, were demonstrated by researcher.

3.7.1 Questionnaire collection

Face to face interview, taking around 15-20 minutes per interviewer , started at the end of farm activities, starting from background information, exposure data, farm data, pesticide use, PPE and end up with sign and symptoms of pesticide exposure. Symptoms and sign of health effects were asked at the time of spraying or within a few hours and to assess the acute effects. All questions were answered by the farmers including observe by the researchers on farm in order to reduce bias from the reporter.

Semen collection and seminal fluid analysis:

- A semen sample was collected by masturbation into a sterile wide-mouth glass container after a recommended period of 2-3 days sexual abstinence.

- The samples were kept in an incubator at 30-36˙ C until liquefaction and subsequently examined. All semen samples were processed and analyzed by qualified personnel based on WHO guidelines (WHO 1992, 1999). (149, 150)
- Volume, pH, sperm concentration, motility, morphology and WBC count were examined and recorded.

Outcome were measured by the adverse effect of pesticides on the male reproductive system especially semen characteristics.

3.7.2 Reproductive hormones collection

- Blood samples were collected in a 10 ml plastic syringe, and keep at room temperature until serum was separated. After centrifugation, the serum was transferred to a new tube, kept at -20˙C, and assayed within 4 weeks. FSH (Follicle-stimulating hormone), LH (Luteinizing hormone) and Testosterone were measured by the electro-chemiluminescence immunoassay "ECCLIA" was intended for use Elecsys and cobas e immunoassay analyzers.

Outcome were measured by Adverse effect of pesticides on the male reproductive system especially serum hormones.

3.7.3 Cholinesterase tests collection

- The blood cholinesterase test measured the effect of exposure to organophosphate and carbamate insecticides.
- Erythrocyte cholinesterase (AChE) showed chronic or long term exposure of OP and CA pesticides and Plasma cholinesterase (PChE) showed a short term exposure indicator to detect acute poisoning early.

Outcome were measured by the effect of exposure to organophosphate and carbamate insecticides (acute & chronic poisoning).

3.7.4 Hands wipe sample collection

Hands wipe samples were collected from ground-nut growing farmers after complete their filed activities (mixing, loading and applying pesticides) before washing or cleaning their hands. If he wore glove, they removed before sampling. Two moisten patches with 40% isopropanol was used to wipe pesticide residue on each hand of farmers thoroughly. Hands wipe sample was kept separately and label farmer's code on square foil. Wipe samples were transfer to zip lock bag separately. All wipe samples were closed, sealed and freeze for transport to laboratory and wait for analysis step (159).

3.8 Data analysis

3.8.1 Statistical Analysis

In this study, analysis of data was done by using SPSS program version 17 for window. In term of describing the general information, knowledge and practice for pesticide usage , seminal analysis, blood hormone level, blood cholinesterase level and health symptoms from exposure to pesticide residues and shown as descriptive statistic (mean, median, standard deviation (SD) , percentage , minimum (Min) and maximum (Max)) was used. The pesticide concentration via dermal exposure was concentrated at both mean and reasonable maximum exposure (RME) at 95th percentile (upper bound) concerning of higher expose ground-nut farmers, including average daily dose (ADD) exposure and Hazard Quotient (HQ) .

Chi-square test was used to find an association between socio-demographic characteristics with knowledge and practices on safe use of pesticide among ground-nut farmers.

Wilcoxon signed rank test was used to compare seminal profile, blood hormonal level and blood cholinesterase level of ground-nut growing male famers between growing and non-growing seasons and this test which makes use the magnitudes of the differences between measurements and a hypothesized location parameter rather than just the signs of the differences.

McNemar’s chi-square test was used compare the reported health symptoms in growing period and non-growing period among male ground-nut farmers.

Multivariate Analysis was used in order to find out risk factors for pesticides exposure effect in each biomarkers level in reproductive health effect and multiple independent variables were personal factors, work-related factors, practice regarding exposure to pesticide residues and protection. Odds ratios of the risk factors for pesticides exposure effect in each biomarkers level in reproductive health effect were calculated by unconditional logistic regressions. Significant level was set at 0.05.

Before Multivariate Analysis, Univariate analysis was done. Univariate Analysis: Associations between independent variables and dependent variables were analyzed by using Pearson's Chi-square test with statistical significant level of less than 0.05. (162). Multivariate Analysis: in order to find out the clear associations between multiple independent variables and a dependent variable at the same time, multivariable regression was used. As the dependent variables such as biomarkers (blood cholinesterase test, blood hormone level, sperm count) are dichotomous outcomes and multiple logistic regressions were used.

For multivariate analysis, the variables which are significant at the level of p value less than 0.2 at bivariate analysis plus those variables that are theoretically important or have been confounders in prior research (even with significance >0.2) will undergo first step of regressions (163). Then, variables with p value of greater than 0.05 were excluded from the analysis to get the final model. Variables included in the model whatever their significance level 1.) if they have been found significant (or confounders) in previous research and 2.) if they are conceptually or theoretically possible explanations for the outcome (163).

3.8.2 Health risk assessment

Health risk assessment for pesticide exposure among ground-nut farmers was evaluated by 4 steps as follows:

1) Hazard identification

In this study, Chlorpyrifos is commonly used by farmers in this area to protect their crops from various pests and that may have an effect on the human body was chosen due to reports of its presence of pesticides residues on humans. This pesticide can enter the human body through the oral route, inhalation, and skin contact, and also pose health problems. Substantial amounts of insecticide can be absorbed through the skin (155). Therefore, this study is to evaluate daily exposure to chlorpyrifos among ground-nut farmers and to assess the health risk of those farmers exposed to chlorpyrifos via dermal pathway.

2) Dose-response assessment

Reference dose (RfD) was an estimation of a daily route exposure to human population who was likely to be without a considerable risk of harmful effect during a lifetime. The unit of RfD in each pesticide is milligram per day (mg/kg-day). Reference dose and toxicity data of each pesticide are shown in Table 3.3.

Table 3. 3 Reference dose and toxicity data of the pesticide

PRs	Classification by Harzard	Cholinesterase inhibitor	RfD (mg/kg-day)
OP group			
Chorpyrifos	II	Yes	0.0003(US EPA,2002) (164)

Classification of active pesticide ingredients by hazard (WHO, 2010) (165) :

II= Moderately hazardous by US EPA.

3) Dermal exposure assessment

The average daily dose (ADD) was applied to estimate the daily dose of ground-nut-growing farmers' exposure for non- carcinogenic effects via dermal route. Some factors in ADD equation were asked from the farmers who applied pesticides by questionnaire. The average daily dose (ADD) was used to exposure non-carcinogenic chemicals as a daily dose on a per-unit-body-weight basis. ADD was a measurement that uses to evaluate the exposure of non-carcinogenic effects. The route-specific mathematical algorithms was used for calculate ADD. For dermal contact with chemicals in soil or water, dermal absorbed average daily dose were evaluate by the equation below (166).

$$\text{ADD dermal (mg/kg} - \text{day)} = \frac{\text{DA event} \times \text{EV} \times \text{ED} \times \text{EF} \times \text{SA}}{\text{BW} \times \text{AT}}$$

eq.3-1

Where:

ADD = average daily dose (mg/kg-day)

DA event = absorbed dose per event (mg/cm2-event)

EV = event frequency (events/day)

ED = exposure duration (years)

EF = exposure frequency (days/years)

SA = skin surface area available for contact (cm2)

BW = body weight (kg)

AT = average time (days) for non-carcinogenic effects, AT = ED in days

According to EPA handbook, total body surface area could be estimated from body weight and body height, which calculated by the equation below (166).

$$\mathbf{SA = a0\ W^{a1}\ H^{a2}} \qquad \textit{eq. 3-2}$$

Where:

SA = surface area (m2)

H = height (cm)

W = weight (kg)

a0, a1, a2 = constant value from US EPA 1997 (166).

The value at 95th percentile level of detected pesticide residues concentration on hands (high-end exposure) was used to examine dermal exposure among ground-nut farmers based on fluctuation of amounts. The 95th percentile of the mean concentration is applied as the average concentration, because it is not possible to know the true mean. Due to the less limited sampling data at a site, uncertainties decrease so that the upper confident level moves closer to the true mean. As a consequence ,the exposure evaluations using either the mean or the upper confident level produce similar results (153).

The two types of exposure estimates now as required for risk assessments, a reasonable maximum exposure (RME) and an average, should both use average concentration. The RME, which is defined as the highest exposure that could reasonably be expected to occur for a given exposure pathway at a site, is intended to account for both uncertainty in the contaminant concentration and variability in exposure parameters. (e.g., exposure frequency, averaging time). It was states that an average estimate of exposure also should be presented in risk assessments (153).

4) Risk characterization

The risk characterization was the last step of human health risk assessment. It carried both qualitative and quantitative data, which was a tool to link with the risk manager or decision makers. The risk characterization was a process that merged and used the relevant technique to analyze the required information from the hazard identification, dose response assessment, and exposure assessment to make risk estimates for the exposure of interest.

OPs were organized as non-carcinogenic pesticide. The reference dose (RfD) was the criterion used in non-carcinogen risk characterization. The individual risks evaluation of non-carcinogenic toxicity was calculated using the hazard quotient (HQ) ratio that reveals the degree of exposure, greater or less than the RfD. If the exposure was more than the RfD, the exposure population may be in danger (158).

$$\text{Hazard Quotient (HQ)} = \frac{\text{Exposure}}{\text{RfD}} \qquad \text{eq. } 3-3$$

Where:

HQ > 1 means adverse non-carcinogenic effect of concern (Risk)

HQ ≤1 means acceptable level (No concern)

Exposure = chemical exposure level or ADD (mg/kg-day)

RfD = reference dose (mg/kg-day)

3.9 Guideline developing for community

According to the results of this study, making guideline books for community who were exposed to pesticides in their working fields. The books expressed how to use pesticides properly and how to wear personal protective equipment. It was given them with demonstration as well as guidelines. It was shown with pictures in Appendix G.

3.10 Ethic consideration

The proposal was approved by the Ethics Committee of Medical Research Center from Myanmar (ERC No: 001414, Ethnics/DMR/2016/035) in May 2, 2016. The researcher was inform the purpose of study and entire the process of study to respondents before running the project. Written consent to participants was voluntarily obtained from all the participants in the study prior to their participation and each participant was paid 5000 Myanmar Kyats ($ 5) to participate.

CHAPTER IV
RESULTS

Kyauk Kan village of Nyaung-U District, Mandalay Region, Myanmar is growing ground-nut as their traditional occupation and one of the famous growing ground-nut zones in Myanmar for long times and had been exposed to pesticides. The study population was focused on individuals living in agricultural communities. This study design provided evaluation of within person changes in the responsive variable across growing and non-growing periods. It consists 2 cross-sectional studies: observational study (Phase I) and laboratory study (Phase II and Phase III).

For observational study (Phase I) included 400 participants with both male and female from 182 households by simple random sampling between total number of 818 people (Male = 366, Female = 452). For laboratory study (Phase II) included 100 male ground-nut farmers from 18 to 49 years of reproductive aged group who are exposed with pesticides in their farms by simple random sampling from the total number of 366 males farmers in this village. For hand wipe test (Phase III) included 30 male ground-nut farmers from 18 to 49 years of reproductive aged group who were chosen chlorpyrifos pesticides in their farms by simple random sampling from the total number of 100 males ground-nut farmers of Phase II.

4.1 Observational Study (Phase I)

4.1.1 General information of the respondents

In this research, there were 400 participants that consisted of 257 men (64.2 %) and 143 women (35.8 %). Most of age them was 38 to 47 years while an average age (±SD) of all was 37.92 ± 8.78 years old. The numbers of married participants were twice than single participants that were 71.2% of married and 24% of single. For education, primary school (Grade 1 to 5) level was 43.8%, which was higher than those who had finished middle school (Grade 6 to 9) level accounted 29.5% and high school (Grade 10 to 11) level counted 15.5% respectively. Average money 1000 kyats = 1 US Dollar and their income level were mostly below 1000000 kyats (<1000 US Dollar) per year was 41.8% and the highest income above 2500001 kyats (>2500 US Dollar) was about 20.8%. The number of smoker and non-smokers were twice in non-smokers that were 71.8% in non-smokers and28.2% of smokers respectively and most of the smokers smoked the burmese traditional cigarettes about 28%. Average smoke per day about 1 to 2 burmese traditional cigarettes among in smokers. Average family member in one family including father and mother that were 5 family members in 29%, 4 family members in 28.2%, 3 family members in 20.5% and the rest were 6, 7 and 8 family members respectively. Among the married couple , counted for total number of children in family and most of them had no children in 29.8%, only 1 child in 22.5% and 2 children in 24.5% respectively (Table 4.1).

Table 4. 1 General information of ground-nut farmers (both male and female) in Kyauk Kan Village, Nyaung U District, Mandalay Region , Myanmar (n = 400)

Characteristics	**Ground-nut Farmers**	
	Number (n = 400)	**Percentage (%)**
Gender		
Male	257	64.2
Female	143	35.8
Age Groups		
Age between 18 to 27 years old	65	16.2
Age between 28 to 37 years old	108	27.0
Age between 38 to 47 years old	163	40.8
Age above 48 years old	64	16.0
Mean age ± SD	37.92 ± 8.78	
Min- Max	18-49	
Marital Status		
Single	96	24.0
Married	285	71.2
Widow	19	4.8
Education level		
Uneducated	7	1.8
Primary School (Grade 1 to 5)	175	43.8

Characteristics	Ground-nut Farmers	
	Number (n = 400)	Percentage (%)
Secondary School (Grade 6 to 9)	118	29.5
High School (Grade 10 to 11)	62	15.5
Diploma	4	1.0
Bachelor's Degree or higher	34	8.5
Income (ground-nut crop/ year)		
(1000 kyats = 1 US Dollar)		
Below 1000000 kyats (<1000 US Dollar)	167	41.8
Between 1000001 - 1500000 kyats (1001-1500 US Dollar)	64	16.0
Between 1500001 - 2500000 kyats (1501-2500 US Dollar)	86	21.5
Above 2500001 kyats (>2500 US Dollar)	83	20.8
Mean income ± SD	1,8230,000±1.27	
Min- Max	200,000-7,000,000kyats	
Smoking Status		
Non-smokers	287	71.8
Smokers	113	28.2
Type of smoke (n = 113 smokers)		
Export cigarette	1	0.2
Burmese Traditional cigar	112	28.0
No. of cigarette/day (n = 113 smokers)		
1 cigarette/day	37	9.2
2 cigarette/day	39	9.8
3 cigarette/day	25	6.2
Above 3 cigarette/day	12	2.9

Characteristics	Ground-nut Farmers	
	Number (n = 400)	Percentage (%)
Family member in one household		
1	4	1.0
2	16	4.0
3	82	20.5
4	113	28.2
5	116	29.0
Above 5	69	17.3
Among the married couple, total no of children		
No Children in the family	119	29.8
1	90	22.5
2	98	24.5
3	61	15.2
Above 3	32	8.0

4.1.2 Ground-nut growing farmers information concern with pesticide exposure

The highest number of pesticide application was ranged below and equal to 10 years that calculated 53.8 % and the second most in between 11 years to 20 years exposure as 43.8% and the rest are 2.5% in equal and above 21 years exposure of pesticides. At this area, mostly they worked in the field at least 4 hours per day with 4-5 persons of teams and also depended on size of cultivation area. The average area cultivated in the past was 12.23 acre (Table 4.2).

There were 3 groups of pesticides that may cause the effect on human body according by choosing and reporting on the presence of pesticides residues on human. They are (1) Organophosphate group included with their trade names : Malathion (carbophos, chemathion, cythion, emmatos, fyfanon, karbofos, kypfos, malaphos, malaspray, malphos) , Chorpyrifos (brodan, detmol, detmolin, dowcol 179, duraban, eradex, killmaster, lock-on, lorsban, loxiran, pyrinex, spannit, stipend, zidil), Profenofos (CGA-15324, curacron, polycron, selecron), EPN (santox), and Phenthoate (cidial, dimephenthoate, dimethenthoate, elsan, papthion, rogodial, tanone) (2) Pyrethriod pesticides (PY group) included Alpha-cypermethrin (bestox, dominex, fastac, fendon) and Cypermethrin (agrothrin, ammo, arrive, avicade, barricade, cymbush, cymperator, cynoff, cyperkill, demon, ectoror, ektomin, equiband, fenom, flectron,clocard, kruel, nurelle, parzon, ripcord, Sherpa, stockade, topple) and (3) Carbamate pesticides (CA group) included Aldicarb (sentry, temik), Carbaryl (crpolin, denapon, dicarbam, hexavin, karbaspray, murvin, patrin,ravyon, septene, sevin, tercyl, tricarnam), Isoprocarb (bay 105807, etrofolan, hytox, MIPC,MIPCIN), Carbofuran (carbodan, carbosip, chinufur, curaterr, furadan, keno, furan, yaltox), Propoxur (aprocarb, baygon, blattanex, PHC, propion, unden, senran, suncide). But in this study, most of the ground-nut famers used organophosphate group and pyrethroids group.

Table 4. 2 Agricultural works and farming characteristics (n = 400)

Area cultivated (Acre) (Mean ± SD)	12.24±8.32
Duration of application /time (Hours) (Mean ± SD) (including mixing , loading and spraying)	4.41±1.52
Years of using pesticides (n(%))	
5-10 years	215(53.8%)
11-20 years	175(43.8%)
21 or more years	10(2.5%)
(Mean ± SD)	10.95±5.02
Duration of application times/week (Mean ± SD)	5.52±1.29
Methods of using to control pest in ground-nut field	
Apply pesticide by themselves	205(51.2%)
Hire someone to apply pesticide	142(35.5%)
Invite relative to help	53(13.2%)

Type of pesticide used in the ground-nut field	
Organophosphate (Chorpyrifos)	111(27.8%)
Pyrethroids (Cypermethrin)	83(20.8%)
Mixed organophosphate and pyrethroids	206(51.5%)

In health information about pesticides exposure in last crop, and the questionnaires ranked about 5 types of answer for each symptoms (never, almost never, during pesticide exposure, shortly after pesticide application and suffered when applied after pesticide and so stopped that pesticide) and restructured as not suffer symptom (never and almost never) and suffer symptom (during pesticide exposure, shortly after pesticide application and suffered when applied after pesticide and so stopped that pesticide). Most of ground-nut farmers in this site suffered dizziness accounted 176 (44.0%) and headache was the second most suffered symptoms calculated 163 (40.8%) and a few 16 (4%) suffered and thought that infertility (Table 4.3).

Table 4. 3 General health information related with pesticide exposure in last crop (n = 400)

Signs and symptoms	**Not Suffer Symptom**	**Suffered Symptom**
Headache	237 (59.2%)	163 (40.8%)
Nausea/Vomiting	361 (29.0%)	39 (9.8%)
Abdomen cramp	372 (93.0%)	28 (7.0%)
Blurred vision	232 (58.0%)	168 (42.0%)
Tearing	304 (76.0%)	96 (24.0%)
Dizziness	234 (56.0%)	176 (44.0%)
Numbness or pins and needles in your hands and feet	357 (89.2%)	43 (10.8%)
Arms and legs weakness	378 (94.5%)	22 (5.5%)
Involuntary twitches or jerks in your arms or legs	379 (94.8%)	21 (5.2%)
Chest tightness	359 (89.8%)	41 (10.2%)
Difficult breathing	347 (86.8%)	53 (13.2%)

Signs and symptoms	Not Suffer Symptom	Suffered Symptom
Loss of libido	384 (96.0%)	16 (4.0%)

4.1.3 Knowledge and Practice Regarding Exposure to Pesticide Residues and Protection

4.1.3.1 Knowledge regarding exposure to pesticide residues and protection

Percentage of knowledge regarding exposure to pesticide residues and protection among ground-nut farmers (both male and female) in 400 participants was illustrated in Table 4.4. The highest item of correct answer (100%) was the question no 8.9. 12 and 13. Whereas, the lowest item of correct answer (53.8%) was the question no7 and half of them knew that "Pesticide can prevent that enter the body by using PPE". All of the participants answered over (50%) in each knowledge items.

Table 4. 4 Percentage of knowledge regarding exposure to pesticide residues and protection among ground-nut farmers (both male and female) in 400 participants

No	Knowledge Item	Correct Answer
1.	Pesticide can cause dangerous of pet in working places.	97.8%
2.	Pesticide can cause adverse effect on human.	96.5%
3.	Environment was damaged by using pesticide.	93.0%
4.	Pesticide can enter the human body accidently.	90.5%
5.	Pesticide can cause toxicity.	82.8%
6.	Pesticide can cause death if pesticide enter the human body accidently.	87.2%
7.	Pesticide can prevent that enter the body by using PPE.	53.8%
8.	Pesticide can enter the human body from environmental media via (water, soil, air)	100.0%
9.	Pesticide can enter the human body via mouth (ingestion) , nose (inhalation) and skin (contact)	100.0%

No	Knowledge Item	Correct Answer
10.	Nervous system, respiratory system, hepatic system, reproductive system and renal system will be damaged by chronic poison of pesticide.	90.8%
11.	Personal Protective Equipment (PPE) required for using pesticide.	76.0%
12.	Eating, smoking and drinking behaviors should be avoided during application of pesticide.	100%
13.	Pesticides should not be sprayed in windy condition, under extreme heat of sun and raining.	100%
14.	Up wind site should be started in spraying pesticides.	96.5%

4.1.3.2 Practice regarding exposure to pesticide residues and protection

Percentage of practice regarding exposure to pesticide residues and protection among ground-nut farmers (both male and female) in 400 participants was illustrated in Table 4.5. The highest item of correct answer (100%) was the question no 6: "Listening from one of them (Neighborhood, Shopkeeper's advice, Advertisement, Agricultural officer and Sales representative) when you decide to purchase pesticide". Whereas, the lowest item of correct answer (7.2%) was the question no 17: "Incinerating method the best for disposing pesticide container".

Table 4. 5 Percentage of practice regarding exposure to pesticide residues and protection among ground-nut farmers (both male and female) in 400 participants

No.	Practice Item	Correct Answer
1.	Using registered pesticides in agriculture.	84.8%
2.	Reading, following and spraying pesticide according to instruction or label.	95%
3.	While spraying pesticide, using personal protective equipment (PPE).	35%
4.	Keeping pesticide with food and water.	69%
5.	Storing pesticide in separate-room (separate/high place/locked box), keeping out of children, animals ,keeping out of food and water source.	96%

No.	Practice Item	Correct Answer
6.	Listening from one of them (Neighborhood, Shopkeeper’s advice, Advertisement, Agricultural officer and Sales representative) when you decide to purchase pesticide.	98%
7.	In general, when mixing pesticide, follow the bottle instruction label.	29.5%
8.	When mixing pesticide, wearing rubber gloves and using stirring stick.	13.2%
9	When mixing pesticide, using personal protective equipment (PPE).	48.2%
10.	Mostly, when mixing and spraying pesticide, contact pesticide with parts of the body.	96.8%
11.	When applying pesticide, wearing with long sleeved shirt and long pants	35.0%
12.	If the participant spill some pesticide on their clothes and body in early morning, changing clothes and clean body immediately	12.2%
13.	After the participant mix and spray pesticide, washing hands and arms immediately and taking a bath after finish work	81.0%
14.	After touching and mixing pesticide, use to clean the body with water and Soap	85.2%
15.	After used pesticide, changing new clothes immediately	14.2%
16.	After that clothes contact with pesticide, wash it immediately	89.0%
17.	Incinerating method the best for disposing pesticide container	7.2%
18.	Never done lunch in paddy field.	44.2%

No.	Practice Item	Correct Answer
19.	Never smoke eat and drink (water) during spraying pesticide.	19.2%

4.1.4 Level of knowledge and practice regarding exposure to pesticide residues and protection

Level of knowledge and practice regarding exposure to pesticide residues and protection among ground-nut farmers (both male and female in 400 participants) were shown in Table 4.6. For knowledge, each correct answer was given 1 score with a total of 31 scores. The scores vary from 0-31 points and will classify into 3 levels as Bloom's cut off point, 60%-80%. Score were classified in 0-10 (less than 60%) as low levels, 11-20 (60-79%) as moderate levels and 21-31 (≥80%) as high levels. The average knowledge score of them was 19.81±5.47 with the range of 4 to 33. The majority of them had moderate level of knowledge (53.5%). For practice, scores range from 0 to 34 and will be classified into 3 levels: good practice 27-34 scores (≥80%), fair practice 16-26 scores (60%-79%) and poor practice 0-15 scores (less than 60%) respectively. The average practice score of them was 13.23±3.11 with the range of 5 to 25. The majority of them had poor level of practice (79.2%).

Table 4. 6 Level of knowledge and practice regarding exposure to pesticide residues and protection among ground-nut farmers

Level of Knowledge and Practice	n (%)	Mean ± SD	Min-Max
Knowledge			
Low (0 to 10 scores)	12(3.0%)		
Moderate (11 to 20 scores)	214(53.5%)	19.81±5.47	4-33
High (21 to 31 scores)	174(43.5%)		
Practice			
Poor (0 to 15 scores)	317(79.2%)		
Fair (16 to 26 scores)	83(20.8%)	13.23±3.11	5-25
Good (27 to 34 scores)	-		

4.1.5 Association between level of knowledge and practice regarding exposure to pesticide residues and protection and socio demographic characteristics (age, sex education and income)

Association between level of knowledge and practice regarding exposure to pesticide residues and protection and socio demographic characteristics (age, sex education and income) were presented in Table 4.7 and 4.8. It was found that there were not statistically significant association between knowledge and practice regarding exposure to pesticide residues and protection and socio demographic characteristics by Pearson Chi-Square test, p value < 0.05 level.

Table 4. 7 Association between level of knowledge regarding exposure to pesticide residues and protection and socio demographic characteristics (age, sex education and income)

Socio-demographic characteristics	**Knowledge Level**							
	Low Knowledge Level (n=12)		**Moderate Knowledge Level (n=214)**		**High Knowledge Level (n=174)**		**Chi-Square**	**P value**
	N	%	N	%	n	%		
Age								
Age between 18 to 27 years old	3	4.6%	29	44.6%	33	50.8%		
Age between 28 to 37 years old	3	2.8%	62	57.4%	43	39.8%		
Age between 38 to 47 years old	5	3.1%	91	55.8%	67	41.1%	3.010	0.390
Age above 48 years old	1	1.6%	32	50.0%	31	48.4%		
Sex								
Male	10	3.9%	139	54.1%	108	42.0%		
Female	2	1.4%	75	52.4%	66	46.2%	0.638	0.425

Socio-demographic characteristics	Knowledge Level							
	Low Knowledge Level (n=12)		Moderate Knowledge Level (n=214)		High Knowledge Level (n=174)		Chi-Square	P value
	N	%	N	%	n	%		
Education								
Uneducated	1	14.3%	6	85.7%	0	-		
Primary School (Grade 1 to 5)	7	4.0%	95	54.3%	73	41.7%		
Secondary School (Grade 6 to 9)	3	2.5%	63	53.4%	52	44.1%	2.551	0.466
High School (Grade 10 to 11)	1	1.6%	29	46.8%	32	51.6%		
Diploma	0	-	4	100%	0	-		
Bachelor's Degree or higher	0	-	17	50%	17	50%		
Income per year								
Below 1000000 kyats (<1000 US dollar)	7	4.2%	88	52.7%	72	43.1%		
Between 1000001 - 1500000 kyats (1001-1500 US Dollar)	0	-	37	57.8%	27	42.2%		
Between 1500001 - 2500000 kyats (1501-2500 US Dollar)	4	4.7%	47	54.7%	35	40.7%	1.073	0.783
Above 2500001 kyats (>2500 US Dollar)	1	1.2%	42	50.6%				

*Significant at the 0.05 level (2-tailed)

Table 4. 8 Association between level of practice regarding exposure to pesticide residues and protection and socio demographic characteristics (age, sex, education and income)

Socio-demographic characteristics	Practice Level					
	Poor Practice Level (n=317)		Fair Practice Level (n=83)		Pearson Chi-Square	P value
	n	%	N	%		
Age						
Age between 18 to 27 years old	56	86.2%	9	13.8%	4.074	0.254
Age between 28 to 37 years old	87	80.6%	21	19.4%		
Age between 38 to 47 years old	122	74.8%	41	25.2%		
Age above 48 years old	52	81.2%	12	18.8%		
Sex						
Male	198	77.0%	59	23.0%	2.130	0.144
Female	119	83.2%	24	16.8%		
Education						
Uneducated	7	100.0%	0	-	5.964	0.113
Primary School (Grade 1 to 5)	143	81.7%	32	18.3%		
Secondary School (Grade 6 to 9)	91	77.1%	27	22.9%		
High School (Grade 10 to 11)	51	82.3%	11	17.7%		
Diploma	2	50%	2	50%		
Bachelor’s Degree or higher	23	67.6%	11	32.4%		

Socio-demographic characteristics	Practice Level					
	Poor Practice Level (n=317)		Fair Practice Level (n=83)		Pearson Chi-Square	P value
	n	%	N	%		
Income per year						
Below 1000000 kyats (<1000 US Dollar)	134	80.2%	33	19.8%		
Between 1000001 - 1500000 kyats (1001-1500 US Dollar)	47	73.4%	17	26.6%		
Between 1500001 - 2500000 kyats (1501-2500 US Dollar)	69	80.2%	17	19.8%	1.574	0.665
Above 2500001 kyats (>2500 US Dollar)	67	80.7%	16	19.3%		

*Significant at the 0.05 level (2-tailed)

4.1.6 Favorable Environment

This favorable environment questionnaires identify upon safe use of pesticide among ground-nut farmers about personal protective equipment were shown in Table 4.9. The most of them answered that personal protective equipment (hat, mask, gloves, protective clothes, rubber boot and apron) were not easily access, afford and buy in this area. Information of PPE (information from different ways such as neighbor, sale man, agricultural sector, media or elder) got from more than one source.

Table 4. 9 Favorable environment (n =400)

Item	n (%)
1. Personal Protective Equipment (PPE) had in this local area.	160 (40%)
2. Access to buy personal protective equipment (PPE) in this area	77 (19.2%)
3. Afford to buy PPE.	102(25.5%)
4. Getting information about PPE from	
(1) Neighbor	126 (31.5%)
(2) Sale man	179 (44.75%)
(3) Agricultural sector.	314 (78.5%)
(4) Media(Radio/TV/	109 (27.25%)

Item	n (%)
Newspaper/Journal)	
(5) Elder	147 (36.75%)

4.2 Laboratory Study (Phase II)

4.2.1 General information of the respondents

In this laboratory study (Phase II), there were 100 participants of male ground-nut farmers who are the age between 18 to 49 years (reproductive age group), sampling from observational study and the average age (±) SD of all was 37.51±9.45 years old. There were also told that all information as well as biological samples and data obtained for the study would remain confidential.

Sample were collected during the main two periods of the agricultural periods: growing period (June to October) in which large quantities of OPs are sprayed (high exposure period), non-growing period (November to April) in which no exposure to OPs. A total of 100 samples were analyzed for each growing and non-growing periods. General information of the respondents data were shown in Table 4.10.

Table 4. 10 Demographic Characteristics of Study Participants in growing and non-growing period in Phase II (n=100)

Characteristics	n (%)
Age (years)	
Mean ± SD (range)	37.5±9.45 (18-49)
Body Mass Index (kg/m²)	
Mean ± SD (range)	18.34±2.14 (16.5-28)
Education	
Primary School Level	34 (34 %)
Middle School Level	39 (39%)
High School Level	19 (19%)
University Level	8 (8%)
Smoking during working time	62 (62%)
Eating and Drinking during working time	76 (76%)

Characteristics	n (%)
Alcohol Habit	45 (45%)
Exposure duration (years) Mean ± SD (range)	 19.2± 7.79 (3-33)
Working days/week Mean ± SD (range)	 6.26±0.53 (5-7)
Working hours/day Mean ± SD (range)	 4.95±0.94 (3-6)
Use of PPE during working	11(11%)
Habit of Washing Hands after working	52(52%)

Observation had done by the primary investigator and three trained interviewer for checking how to follow, store and use or not in their area even asked about questionnaires and this might be counter checked and all these data will be helped and relevance to the questionnaires and the result shown in Table 4.11. According to the observation checked, (100%) of all the respondents were using registered pesticide and all the bottle of pesticides were included on package but they did not stored properly for pesticide bottles in their homes. Obviously (67%) of them were keeping pesticide near with food containers and all of (100%) were not using personal protective equipment during mixing and spraying pesticides in their fields.

Table 4. 11 Observation Check List for Agricultural workers (n= 100)

Observation Check Lists	Yes	No
Currently use of registered pesticide	100(100%)	-
Instruction is included on package	100(100%)	-
Do you understand instruction on package of pesticide	92(92%)	8(8%)
Pesticide is placed safety 1.separate room/high/ in locked room 2.keep with agricultural equipment 3. keep with food	 - 8(8%) 67(67%)	 100(100%) 92(92%) 33(33%)
Sufficient amount of water and soap to wash hand and body	-	100(100%)

Observation Check Lists	Yes	No
Use of Personal Protective Equipment (PPE)	-	100(100%)

4.2.2 Semen collection and seminal fluid analysis

All semen samples were processed and analyzed by qualified personnel based on WHO guidelines (WHO 1992, 1999) (149, 150). Semen analysis included: seminal volume, pH, viscosity, motility, morphology and sperm count were examined and recorded. Table 4.12 shows that the results of seminal analysis in growing and non-growing period. If any discrepancy and error were noticed, the semen analysis including the count was repeated for two or three time to assure the accuracy. All of the variables of semen analysis were higher in abnormal at growing period than the non-growing period because of that, in growing period, the ground-nut farmers grew the farming and used the pesticides for their farms and also exposed with pesticides. But in non-growing period, the ground-nut farmers had not been exposed to pesticides. By this way, in non-growing period, the results in each parts of the variables of semen analysis were increased in the normal than the growing period. A Shapro-Wilk's test ($p>0.05$) (167) and a visual inspection of their histograms, normal Q-Q plots and box plots showed that most of the variables of semen analysis were not normally distributed and so used as median for all of the variables of semen analysis in both growing and non-growing period and normality test resulted was shown in Appendix H.

Table 4. 12 Proportion of respondents who were exposed to OPs with semen quality in Growing and Non- Growing Period (N = 100)

Seminal Profile	Growing Period			Non-Growing Period			Ref; (WHO)
	Normal n (%)	Abnormal n (%)	Median (Min-Max)	Normal n (%)	Abnormal n (%)	Median (Min-Max)	
Volume	25(25%)	75(75%)	1.65 (0.2-4.5)	48(48%)	52(52%)	1.60 (0.5-6.4)	(2.0-5cc)
pH	58(58%)	42(42%)	(6.0-8.5)	96(96%)	4(4%)	(7-8.5)	(7.2-7.8)
Viscosity	23(23%)	77(77%)	45.0 (15-120)	58(58%)	42(42%)	30.0 (10-120)	Within 30 minutes
Motility	9(9%)	91(91%)	50.0 (0-90)	13(13%)	87(87%)	30.0 (3-80)	>80% motile

Seminal Profile	Growing Period			Non-Growing Period			Ref; (WHO)
	Normal n (%)	Abnormal n (%)	Median (Min-Max)	Normal n (%)	Abnormal n (%)	Median (Min-Max)	
Morphology	5(5%)	95(95%)	67.5 (0-100)	9(9%)	91(91%)	75.0 (40-100)	The tail takes up about 90% of total length
Sperm Count	23(23%)	77(77%)	30.5 (0-1176)	54(54%)	46(46%)	64.0 (8-464)	(60-150)x10^6

Normozoospermia means that Normal count, motility and morphology, **Oligozoospermia** means that below the lower reference limit, **Necrozoospermia** means that low percentage of live, high percentage of immotile and **Azoospermia** means that No spermatozoa in the ejaculate (149, 150)and Table 4.13 shows the diagnosis of seminal analysis.

Table 4. 13 Diagnosis for sperm analysis in Growing and Non-Growing Period (n = 100)

Diagnosis	**Growing Period**	**Non-Growing Period**
Normozoospermia	24(24.00%)	54(54.00%)
Oligozoospermia	74(74.00%)	46(46.00%)
Necrozoospermia	1(1.00%)	-
Azoospermia	1(1.00%)	-

4.2.2.3 Comparison of seminal profile level between growing and non-growing period

The seminal profile level of ground-nut farmers between growing and non-growing periods were compared by Wilcoxon signed rank test. The analysis revealed the statistically significant difference of all seminal profile level such as pH, viscosity, motility, morphology and sperm count in both seasons except the sperm volume level. Among seminal profile levels (pH, viscosity, motility, morphology and sperm count) in growing period were significantly higher than those in non-growing period (p value < 0.05). Comparison of seminal profile level between growing and non-growing periods by Wilcoxon signed rank test in Table 4.14.

Table 4. 14 Comparison of seminal profile level between growing and non-growing periods by Wilcoxon Signed Rank Test (n= 100)

Seminal Profile	**Growing Period**	**Non-growing Period**	Wilcoxon statistic (z value)	**P Value**
Sperm Volume (2.0-5cc)				
Median	1.65	1.60		
Minimum- Maximum	0.2-4.5	0.50-6.40	-1.095	0.274
pH (7.2-7.8)				
Minimum-Maximum	6.00-5.50	7.00-8.50	-6.076	0.000*
Viscosity (Within 30 minutes)				
Median	45.00	30.00		
Minimum-Maximum	15.00-120.00	10.00-120.00	-4.858	0.000*
Motility (>80% motile)				
Median	50.00	30.00		
Minimum-Maximum	0.00-90.00	3.00-80.00	-2.838	0.005*
Morphology (The tail takes up about 90% of total length)				
Median	67.50	75.00		
Minimum-Maximum	0.00-100.00	40.00-100.00	-4.338	0.000*
Sperm Count $((60\text{-}150)\times 10^{6})$				
Median	30.50	64.00		
Minimum-Maximum	0.00-1176.00	8.00-464.00	-4.852	0.000*

*Significance at 0.05 level (2 tailed) by Wilcoxon Signed Rank Test

4.2.3 Blood Collection and Reproductive Hormone Assay

The normal value of the reproductive hormones in the ground-nut farmers are as follows: FSH (Follicle-stimulating hormone) is 1.5-12.4mIU/ml, LH(Luteinizing hormone) is 1.7-8.6mIU/ml and Testosterone is 0.2-1.4 ng/ml respectively (151).Low and high level determined as abnormal for each hormonal levels. Table 4.15 gives the results of determining the blood hormonal level of ground-nut growing male farmers for finding out the effect of chemicals on male reproductive function who are chronically exposed to different kinds of organophosphate pesticides in both growing and non-growing period. A Shapro-Wilk's test (p>0.05) (167) and a visual inspection of their histograms, normal Q-Q plots and box plots showed that all of the variables of hormonal levels were not normally distributed and so used as median for all of the variables of hormonal level in both growing and non-growing period and normality test resulted was shown in Appendix H.

Table 4. 15 Proportion of respondents who were exposed to OPs with serum hormonal level in Growing and Non- Growing Period (n= 100)

Serum Hormone		**Growing Period**		**Non-Growing Period**	
		n (%)	**Median (Min-Max)**	**n (%)**	**Median (Min-Max)**
Follicle Stimulating Hormone (1.5-12.4 mIU/ml)	Low FSH	2 (2%)	4.34 (1.33-27.46)	2(2%)	4.80 (1.45-27.8)
	Normal FSH	92(92%)		90(90%)	
	High FSH	6(6%)		8(8%)	
Luteinizing Hormone (1.7-8.6 mIU/ml)	Low LH	-	6.79 (2.68-18.11)	1(1%)	6.64 (1.29-21.7)
	Normal LH	68(68%)		75(75%)	
	High LH	32(32%)		24(24%)	
Testosterone (0.2-1.4 ng/ml)	Low Testosterone	6(6%)	5.17 (1.84-11.41)	6(6%)	5.45 (2.15-14.4)
	Normal Testosterone	88(88%)		82(82%)	
	High Testosterone	6(6%)		12(12%)	

4.2.3.1 Comparison of blood hormone level between growing and non-growing period

The blood hormone level of ground-nut farmers between growing and non-growing periods were compared by Wilcoxon signed rank test. The analysis revealed the statistically significant difference of blood hormone level in both seasons. The testosterone secreted by the testes in response to LH has the reciprocal effect of inhibiting anterior pituitary secretion of LH. Whenever secretion of testosterone becomes too great, this automatic negative feedback effect, operating through the hypothalamus and anterior pituitary gland, reduces the testosterone secretion back toward the desired operating level. When the seminiferous tubules fail to produce sperm, secretion of FSH by the anterior pituitary gland increases markedly. Conversely, when spermatogenesis proceeds too rapidly, pituitary secretion of FSH diminishes (32). Among blood hormone levels of FSH and Testosterone level in growing period were significantly lesser than those in non-growing period (p value < 0.05). Comparison of blood hormone level between growing and non-growing periods by Wilcoxon signed rank test were presented in Table 4.16.

Table 4. 16 Comparison of blood hormone level between growing and non-growing periods by Wilcoxon signed rank test (n = 100)

Serum Hormone	**Growing Period**	**Non-growing Period**	Wilcoxon statistic (z value)	**P Value**
Follicle Stimulating Hormone (1.5-12.4 mIU/ml) Median Minimum-Maximum	 4.35 1.33-27.46	 4.81 1.45-27.80	 -2.326	 0.020*
Luteinizing Hormone (1.7-8.6 mIU/ml) Median Minimum-Maximum	 6.79 2.68-18.11	 6.65 1.29-21.07	 -0.951	 0.342
Testosterone (0.2-1.4 ng/ml) Median Minimum-Maximum	 5.17 1.84-11.41	 5.46 2.15-14.36	 -2.876	 0.004*

*Significance at 0.05 level (2 tailed) by Wilcoxon Signed Rank Tes

4.2.4 Blood Cholinesterase testing

A Shapro-Wilk's test ($p>0.05$) (167) and a visual inspection of their histograms, normal Q-Q plots and box plots showed that all of the variables of blood cholinesterase levels (AChE, HAChE and PChE) were not normally distributed and so used as median for all of the variables of blood cholinesterase levels in both growing and non-growing period and normality test resulted was shown in Appendix H. For interpretation of ChE results, The ChE values were classified by using median values for cut-off points into 2 levels such as abnormal and normal level (168). If the value was equal to or less than 3.15 U/ml for AChE, **25.4** U/g Hgb for HAChE, and 1.61 U/ml for PChE in growing period and if the value was equal to or less than 3.13 U/ml for AChE, **27.5** U/g Hgb for HAChE, and 1.28 U/ml for PChE in non-growing period, it was considered "abnormal level". It was assumed that participants could possibly have pesticide poisoning. If the value of AChE, HAChE and PChE was more than 3.15, **25.4** and 1.61 U/ml in growing period and 3.13, **27.5**, and 1.28 U/ml in non-growing period respectively, it indicated "normal level". For measuring the effect of exposure to organophosphate pesticides (acute & chronic poisoning) among the farmers by blood cholinesterase monitoring and results show in Table 4.17.

Table 4. 17 Proportion of respondents who were exposed to OPs with blood cholinesterase monitoring in Growing and Non- Growing Period (n = 100)

Blood cholinesterase monitoring		**Growing Period**		**Non- Growing Period**	
		Frequency (%)	**Median (Min-Max)**	**Frequency (%)**	**Median (Min-Max)**
Acetyl cholinesterase: AChE (U/ml)	Normal	39(39%)	3.15 (2.01 – 5.46)	50(50%)	3.13 (1.67-4.69)
	Abnormal	61(61%)		50(50%)	

Blood cholinesterase monitoring		Growing Period		Non- Growing Period	
		Frequency (%)	**Median (Min-Max)**	**Frequency (%)**	**Median (Min-Max)**
Hemoglobin adjusted acetyl cholinesterase: HAChE (U/g Hgb)	Normal	50(50%)	25.4 (14 – 40.30)	49(49%)	27.5 (18.80-42.40)
	Abnormal	50(50%)		51(51%)	
Plasma cholinesterase: PChE (U/ml)	Normal	43(43%)	1.61 (0.70 – 3.28)	50(50%)	1.28 (0.01-3.24)
	Abnormal	57(57%)		50(50%)	

4.2.4.1 Comparison of seminal profile level between growing and non-growing period

The blood cholinesterase level of ground-nut farmers between growing and non-growing periods were compared by Wilcoxon signed rank test. The analysis revealed the statistically significant difference of blood cholinesterase level in both seasons. Among blood cholinesterase levels of plasma cholinesterase HAChE and (PChE) in growing period were significantly higher than those in non-growing period (p value < 0.05). Comparison of blood cholinesterase level between growing and non-growing periods by Wilcoxon signed rank test were presented in Table 4.18

Table 4. 18 Comparison of blood cholinesterase level between growing and non-growing periods by Wilcoxon signed rank test (n = 100)

Blood cholinesterase monitoring	**Growing Period**	**Non-Growing Period**	**Wilcoxon statistic (z value)**	**P Value**
Acetyl cholinesterase: AChE (U/ml)				
Median	3.15	3.13		
Minimum-Maximum	2.01-5.46	1.67-4.69	-1.700	0.089
Hemoglobin adjusted acetyl cholinesterase: HAChE (U/ml)				
Median	25.4	27.5		
Minimum-Maximum	14 – 40.30	18.80-42.40	-3.462	0.001*
Plasma cholinesterase: PChE (U/ml)				
Median	1.61	1.28		
Minimum-Maximum	0.70-3.28	0.01-3.24	-5.269	0.000*

*Significance at 0.05 level (2 tailed) by Wilcoxon Signed Rank Test

4.2.5 Health Symptoms from Exposure to Pesticide Residues

4.2.5.1 Health symptoms between growing and non-growing period

The reported subjected sign and symptoms related to common pesticide exposure from ground-nut farmers in growing and non-growing period were shown in Table 4.19. Of the (100) ground-nut farmers completed data collection and clinical examination in growing periods. It was reported that the most frequent symptoms in growing period which was exposed with pesticide residues were blurred vision (27%), followed by dizziness and headache as (13%) and (12%) respectively. General symptoms such as sweating and weakness, itching of skin were (6%) and (3%) each. No obviously health effect from exposure to pesticide exposure during growing period by interviewing and clinical examination and the main symptom for reproductive system mean that loss of libido was suffered in (4%) among 100 ground-nut farmers.

Reports of health symptoms after spraying and within the day of spraying time in growing period and no exposure for pesticide but is there any suffering health symptoms happened after harvesting from the work in non-growing period by interviewing and clinical assessment between the same 100 male ground-nut farmers and the data of symptoms such as blurred vision (10%), headache (7%), dizziness (5%) and weakness (5%). All of these subjected signs and symptoms were not worsen in both two seasons and a few of symptoms were suffered more in growing period than non-growing period and for reproductive system, loss of libido also decreased (2%) only in non-growing period than growing period because in that duration, the ground-nut farmers did not exposed to pesticide residues.

Table 4. 19 Subjective signs and symptoms in growing and non-growing period among male ground-nut farmers (n = 100)

Health Symptoms	**Growing Period**		**Non-Growing Period**	
	Normal	**Abnormal**	**Normal**	**Abnormal**
General Symptoms:				
Weakness	97	3	95	5
Itching of skin	97	3	99	1
Sweating	94	6	96	4
Gastrointestinal:				
Nausea	95	5	98	2
Vomiting	99	1	100	-
Pain in abdomen	99	1	99	1
Loose motion	99	1	98	2
Central Nervous System:				
Headache	88	12	93	7
Dizziness	87	13	95	5
Irritability	99	1	100	-
Paraesthesia	100	-	100	-
Blurred vision	73	27	90	10
Mental confusion	100	-	100	-
Convulsion	100	-	100	-
Hallycination	100	-	100	-
Unconsciousness	100	-	100	-
Physical Signs:				
Pupilalry constriction	99	1	100	-

Health Symptoms	Growing Period		Non-Growing Period	
	Normal	Abnormal	Normal	Abnormal
Conjunctival redness	95	5	98	2
Pallor	91	9	95	5
Cyanosis	100	-	100	-
Hyperpyrexia	100	-	100	-
Cardiovascular System				
Ausculation	100	-	100	-
Respiratory System				
Auscultation	100	-	100	-
Gastrointestinal Tract				
Liver	98	2	99	1
Spleen	100	-	99	1
Skin:				
Redness	93	7	97	3
Swelling	100	-	100	-
Dermatitis	95	5	98	2
Tone				
Upper limb	100	-	100	-
Lower limb	96	4	99	1
Loss of biceps jerk	100	-	100	-
Loss of triceps jerk	100	-	100	-
Loss of supinator jerk	100	-	100	-
Loss of ankle jerk	100	-	100	-
Loss of knee jerk	98	2	99	1
Reproductive-System				
Infertility	96	4	98	2
Mental Assessment				
Stress Test*(WHO Guide Line)	97	3	99	1

*Stress test questionnaires and scoring shown in Appendix C.

4.2.5.2 Comparison of health symptoms between growing and non-growing period

Comparison of reported health symptoms in growing period and non-growing period among male ground-nut farmers by McNemar's chi-square test were presented in Table 4.20. Reporting health symptoms , it was found that there were significant difference in proportion of dizziness and blurred vision under central nervous system between two periods (p-value <0.05) whereas there was no significant differences in proportion of other symptoms of systems were found.

Table 4. 20 Comparison of reported health symptoms in growing and non-growing period among male ground-nut farmers by McNemar's chi-square test (n = 100)

Health symptoms	**Growing period n (frequency)**	**Non-growing period n (frequency)**	**p-value**
General Symptoms:			
Weakness	3	5	0.500
Itching of skin	3	1	0.500
Sweating	6	4	0.500
Gastrointestinal:			
Nausea	5	2	0.250
Vomiting	1	-	-[a]
Pain in abdomen	1	1	1.000
Loose motion	1	2	1.000
Central Nervous System:			
Headache	12	7	0.062
Dizziness	13	5	0.008*
Irritability	1	-	1.000
Paresthesia	-	-	-[a]
Blurred vision	27	10	0.000*
Mental confusion	-	-	-[a]
Convulsion	-	-	-[a]
Hallucination	-	-	-[a]
Unconsciousness	-	-	-[a]
Physical Signs:			
Pupillary-constriction	1	-	-[a]
Conjunctival redness	5	2	0.250

Pallor	9	5	0.125
Cyanosis	-	-	-[a]
Hyperpyrexia	-	-	-[a]
Cardiovascular System			
Auscultation	-	-	-[a]
Respiratory System			
Auscultation	-	-	-[a]
Gastrointestinal Tract			
Liver	2	1	1.000
Spleen	-	1	-[a]
Skin:			
Redness	7	3	1.000
Swelling	-	-	-[a]
Dermatitis	5	2	0.250
Tone			
Muscle bulk	-	-	-[a]
Fasciculation	-	-	-[a]
Tremors	2	1	1.000
Upper limb	-	-	-[a]
Lower limb	4	1	0.250
Loss of biceps jerk	-	-	-[a]
Loss of triceps jerk	-	-	-[a]
Loss of supinator jerk	-	-	-[a]
Loss of ankle jerk	-	-	-[a]
Loss of knee jerk	2	1	1.000
Reproductive-System			

Infertility	4	2	1.000
Mental Assessment			
Stress Test Ϯ (WHO Guide Line)	3	1	0.500

*Significance at the 0.05 level (2 tailed)
[a] Not computed
Ϯ Stress test questionnaires and scoring shown in Appendix.

4.2.6 Factor associated between biomarkers (Blood Cholinesterase Monitoring , Blood Hormonal Assay and Sperm Count) and work related factors in Growing Period and Non-Growing Period

For this study, dependent variables are biomarkers such as AChE, HAChE, PChE, FSH, LH, Testosterone and Sperm Count between growing and non-growing period with age, education, working hours, working years, smoking at the work, eating and drinking at working, alcohol drinking at works, use of PPE and washing hands after the working for finding out the relation between each or not. There were several independent variables but only choose these nine variables because these factors were used as most common independent related factors by literature reviewing ((137) (169) (170) (168) (28) (12)). For age variable, used as mean value for cut of point from table 4.10 of demographic characteristics of study participants between growing and non-growing period in Phase II and for working hours was used as above 5 hours and working years used as above 10 years as the reference from Khin Maung Nyunt studied on assessment of knowledge and effects of pesticides on the farm workers, Myanmar (28).

4.2.6.1 Factor associated between Blood Cholinesterase Monitoring and Work Related Factors in Growing Period and Non-Growing Period

4.2.6.1.1 Factor associated Acetyl cholinesterase: AChE in Growing Period

Factor associated between blood cholinesterase monitoring (AChE) in growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of

PPE and washing hands after working in growing period by bivariate analysis and shows in Table 4.21.

Table 4. 21 Bivariate analysis of work related factors and AChE abnormal in growing period (n = 100)

Acetyl cholinesterase (AChE) (Growing period)					
Variables	**Normal AChE**	**Abnormal AChE**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	23(23%)	29(29%)	1	-	
≥ 40 years old	16(16%)	32(32%)	1.586	0.704-3.573	0.264
Education					
< middle school level	13(13%)	21(21%)	1	-	
≥middle school level	26(26%)	40(40%)	0.952	0.407-2.228	0.910
Working Hours					
<5 hours	11(11%)	15(15%)	1	-	
≥5 hours	28(28%)	46(46%)	1.205	0.485–2.990	0.688
Working Years					
<10 years	7(7%)	8(8%)	1	-	
≥10 years	32(32%)	53(53%)	1.449	0.480–4.376	0.509
Smoking at the work					
No	19(19%)	19(19%)	1	-	
Yes	20(20%)	42(42%)	2.100	0.916–4.813	0.077
Eating/drinking at the work					
No	6(6%)	18(18%)	1	-	
Yes	33(33%)	43(43%)	0.434	0.155–1.216	0.107
Alcohol drinking at the work					

No	18(18%)	37(37%)	1	-	
Yes	21(21%)	24(24%)	0.556	0.247–1.253	0.155
Use of PPE					
Use of PPE	5(5%)	6(6%)	1	-	
Non-use of PPE	34(34%)	55(55%)	1.348	0.382–4.760	0.642
Washing Hands After Working					
Hand washings	21(21%)	31(31%)	1	-	
No Hand washings	18(18%)	30(30%)	1.129	0.505–2.526	0.768

Using X^2 test; 'p' significant at α = 0.05 level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and AChE abnormal level in growing period. There was no significant association between age, education, working hours, working years, smoking and eating/drinking and alcohol drinking at the work , use of PPE & washing hands after working and AChE abnormal level in growing period. It could be found that only p-value < 0.2 level was found in smoking and eating/drinking and alcohol drinking at the work and AChE abnormal level in growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including three risk factors namely history of smoking and eating/drinking and alcohol drinking at the work. So multiple logistic regression analysis was done exploring all possible risk factors for history of smoking and eating/drinking and alcohol drinking at the work with AChE abnormal in growing period (p-value were 0.077, 0.107 and 0.155) respectively were shown in Table 4.22.

Table 4. 22 Multiple logistic regressions of work related factors and AChE abnormal in growing period (n = 100)

Acetyl cholinesterase (AChE) (Growing period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Smoking at the work			
No	1	-	
Yes	2.929	1.172–7.321	0.021*
Eating/drinking at the work			
No	1	-	
Yes	0.300	0.097–0.921	0.035*
Alcohol drinking at the work			
No	1	-	
Yes	0.498	0.212–1.172	0.111

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; 'p' significant at $\alpha = 0.05$

According to the study results, history of smoking and eating/drinking at the work are significantly associated with AChE abnormal in growing period (p-value < 0.05). Adjusted the factors, the odd of history of smoking group participants were 2.93 times more effect to get AChE abnormal than non-smoking group , the odd of history of eating/ drinking group participants were 0.30 lesser chance to have abnormal AChE level compared to no history of eating and drinking group participants at the work , the odd of having history of alcohol drinking group participants were 0.49 times lesser to get AChE abnormal compared to no history of alcohol drinking group participants in this study.

4.2.6.1.2 Factor associated Acetyl cholinesterase: AChE in Non-Growing Period

Factor associated between blood cholinesterase monitoring (AChE) in non-growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in non-growing period by bivariate analysis and shows in Table 4.23.

Table 4. 23 Bivariate analysis of work related factors and AChE abnormal in non-growing period (n = 100)

Acetyl cholinesterase (AChE) (Non-growing period)					
Variables	**Normal AChE**	**Abnormal AChE**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	27(27%)	25(25%)	1	-	
≥ 40 years old	23(23%)	25(25%)	1.174	0.535-2.574	0.689
Education					
< middle school level	18(18%)	16(16%)	1	-	
≥middle school level	32(32%)	34(34%)	1.195	0.522-2.737	0.673
Working Hours					
<5 hours	14(14%)	12(12%)	1	-	
≥5 hours	36(36%)	38(38%)	1.231	0.503–3.016	0.648
Working Years					
<10 years	10(10%)	5(5%)	1	-	
≥10 years	40(40%)	45(45%)	2.250	0.709–7.141	0.161
Smoking at the work					
No	22(22%)	16(16%)	1	-	
Yes	28(28%)	34(34%)	1.670	0.739–3.774	0.216
Eating/drinking at the work					
No	10(10%)	14(14%)	1	-	
Yes	40(40%)	36(36%)	0.643	0.254–1.626	0.349

Alcohol drinking at the work					
No	27(27%)	28(28%)	1	-	
Yes	23(23%)	22(22%)	0.922	0.419–2.028	0.841
Use of PPE					
Use of PPE	4(4%)	7(7%)	1	-	
Non-use of PPE	46(46%)	43(43%)	0.534	0.146–1.954	0.338
Washing Hands After Working					
Hand washings	26(26%)	26(26%)	1	-	
No Hand washings	24(24%)	24(24%)	1.000	0.456–2.192	1.000

Using X^2 test; 'p' significant at $\alpha = 0.05$ level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and AChE abnormal level in non-growing period. There was no significant association between age, education, working hours, working years, smoking and eating/drinking and alcohol drinking at the work, use of PPE & washing hands after working and AChE abnormal level in non-growing period. It could be found that only p-value < 0.2 level was found only in working years and AChE abnormal level in non-growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, working hours, working years, smoking and eating/drinking and alcohol drinking at the work use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including only one risk factor namely working years. So multiple logistic regression analysis was done exploring all possible risk factors for working years with AChE abnormal in non-growing period (p-value were 0.161) was shown in Table 4.24.

Table 4. 24 Multiple logistic regressions of work related factors and AChE abnormal in non-growing period (n = 100)

Acetyl cholinesterase (AChE) (Non-growing period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Working Years			
<10 years	1	-	
≥10 years	2.250	0.709–7.141	0.169

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; ‘p’ significant at $\alpha = 0.05$

According to the study results, working years is not significantly associated with AChE abnormal in non-growing period. Adjusted the factor, the odd of working more and equal to 10 years group participants were 2.25 times more effect to get AChE abnormal than less than 10 years group participants among non-growing period in this study.

4.2.6.1.3 Factor associated Hemoglobin adjusted acetyl cholinesterase: HAChE in Growing Period

Factor associated between blood cholinesterase monitoring (HAChE) in growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in growing period by bivariate analysis and shows in Table 4.25.

Table 4. 25 Bivariate analysis of work related factors and HAChE abnormal in growing period (n = 100)

Hemoglobin Adjusted Acetyl cholinesterase (HAChE) (Growing period)					
Variables	**Normal HAChE**	**Abnormal HAChE**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	25(25%)	27(27%)	1	-	
≥ 40 years old	25(25%)	23(23%)	0.852	0.388-1.868	0.689
Education					
< middle school level	17(17%)	17(17%)	1	-	
≥middle school level	33(33%)	33(33%)	1.000	0.437-2.288	1.000
Working Hours					
<5 hours	15(15%)	11(11%)	1	-	
≥5 hours	35(35%)	39(39%)	1.519	0.617–3.745	0.362
Working Years					
<10 years	9(9%)	6(6%)	1	-	
≥10 years	41(41%)	44(44%)	1.610	0.527–4.920	0.401
Smoking at the work					
No	19(19%)	19(19%)	1	-	
Yes	31(31%)	31(31%)	1.000	0.446–2.242	1.000
Eating/drinking at the work					
No	12(12%)	12(12%)	1	-	
Yes	38(38%)	38(38%)	1.000	0.399–2.504	1.000
Alcohol drinking at the work					
No	25(25%)	30(30%)	1	-	
Yes	25(25%)	20(20%)	0.667	0.302–1.472	0.316

Use of PPE					
Use of PPE	4(4%)	7(7%)	1	-	
Non-use of PPE	46(46%)	43(43%)	0.534	0.146–1.954	0.338
Washing Hands After Working					
Hand washings	26(26%)	26(26%)	1	-	
No Hand washings	24(24%)	24(24%)	1.000	0.456–2.192	1.000

Using X^2 test; 'p' significant at $\alpha = 0.05$ level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and HAChE abnormal level in growing period. There was no significant association between age, education, working hours, working years, smoking and eating/drinking and alcohol drinking at the work , use of PPE & washing hands after working and HAChE abnormal level in growing period. According to bivariate logistic regressions, all the p-value results were more than 0.2 level and so that HAChE in growing period could not calculate for binary logistic regression as multivariate analysis with age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in this study.

4.2.6.1.4 Factor associated Hemoglobin adjusted acetyl cholinesterase: HAChE in Non-Growing Period

Factor associated between blood cholinesterase monitoring (HAChE) in non-growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in non-growing period by bivariate analysis and shows in Table 4.26.

Table 4. 26 Bivariate analysis of work related factors on HAChE abnormal in non-growing period (n = 100)

Hemoglobin Adjusted Acetyl cholinesterase (HAChE) (non-growing period)					
Variables	**Normal HAChE**	**Abnormal HAChE**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	26(26%)	26(26%)	1	-	
≥ 40 years old	23(23%)	25(25%)	1.087	0.496-2.383	0.835
Education					
< middle school level	16(16%)	18(18%)	1	-	
≥middle school level	33(33%)	33(33%)	0.889	0.388-2.035	0.780
Working Hours					
<5 hours	15(15%)	11(11%)	1	-	
≥5 hours	34(34%)	40(40%)	1.604	0.651–3.955	0.303
Working Years					
<10 years	5(5%)	10(10%)	1	-	
≥10 years	44(44%)	41(41%)	0.466	0.147–1.478	0.188
Smoking at the work					
No	19(19%)	19(19%)	1	-	
Yes	30(30%)	32(32%)	1.067	0.476–2.392	0.876
Eating/drinking at the work					
No	10(10%)	14(14%)	1	-	
Yes	39(39%)	37(37%)	0.678	0.268–1.714	0.410
Alcohol drinking at the work					
No	25(25%)	30(30%)	1	-	
Yes	24(24%)	21(21%)	0.729	0.331–1.607	0.433

Use of PPE					
Use of PPE	4(4%)	7(7%)	1	-	
Non-use of PPE	45(45%)	44(44%)	0.559	0.153–2.044	0.374
Washing Hands After Working					
Hand washings	26(26%)	26(26%)	1	-	
No Hand washings	23(23%)	25(25%)	1.087	0.496–2.383	0.835

Using X^2 test; 'p' significant at α = 0.05 level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and HAChE abnormal level in non-growing period. There was no significant association between age, education, working hours, working years, smoking and eating/drinking and alcohol drinking at the work, use of PPE & washing hands after working and HAChE abnormal level in non-growing period. It could be found that only p-value < 0.2 level was found only in working years and HAChE abnormal level in non-growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, working hours, working years, smoking and eating/drinking and alcohol drinking at the work use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including only one risk factor namely working years. So multiple logistic regression analysis was done exploring all possible risk factors for working years with HAChE abnormal in non-growing period (p-value were 0.188) was shown in Table 4.27.

Table 4. 27 Multiple logistic regressions of work related factors and HAChE abnormal in non-growing period (n = 100)

Hemoglobin Adjusted Acetyl cholinesterase (HAChE) (Non-growing period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Working Years			
<10 years	1	-	
≥10 years	0.466	0.147–1.478	0.195

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; ‘p’ significant at $\alpha = 0.05$

According to the study results, working years is not significantly associated with HAChE abnormal in non-growing period. Adjusted the factor, the odd of working more and equal to 10 years group participants were 0.47 times lesser chance to get HAChE abnormal than less than 10 years group participants among non-growing period in this study.

4.2.6.1.5 Factor associated Plasma cholinesterase: PChE in Growing Period

Factor associated between plasma cholinesterase monitoring (PChE) in growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in non-growing period by bivariate analysis and shows in Table 4.28.

Table 4. 28 Bivariate analysis of work related factors and PChE abnormal in growing period (n = 100)

Plasma Cholinesterase (PChE) (Growing Period)					
Variables	**Normal PChE**	**Abnormal PChE**	**Uni-variate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	21(21%)	31(31%)	1	-	
≥ 40 years old	22(22%)	26(26%)	0.801	0.362-1.770	0.582
Education					
< middle school level	16(16%)	18(18%)	1	-	
≥middle school level	27(27%)	39(39%)	1.284	0.558-2.954	0.556
Working Hours					
<5 hours	13(13%)	13(13%)	1	-	
≥5 hours	30(30%)	44(44%)	1.467	0.598–3.600	0.402
Working Years					
<10 years	5(5%)	10(10%)	1	-	
≥10 years	38(38%)	47(47%)	0.618	0.195–1.964	0.412
Smoking at the work					
No	19(19%)	19(19%)	1	-	
Yes	24(24%)	38(38%)	1.583	0.700-3.580	0.268
Eating/drinking at the work					
No	12(12%)	12(12%)	1	-	
Yes	31(31%)	45(45%)	1.452	0.578–3.649	0.427
Alcohol drinking at the work					
No	27(27%)	28(28%)	1	-	
Yes	16(16%)	29(29%)	1.748	0.779–3.919	0.174

Use of PPE					
Use of PPE	4(4%)	7(7%)	1	-	
Non-use of PPE	39(39%)	50(50%)	0.733	0.200-2.682	0.637
Washing Hands After Working					
Hand washings	19(19%)	33(33%)	1	-	
No Hand washings	24(24%)	24(24%)	0.576	0.259–1.280	0.174

Using X^2 test; ‘p’ significant at α = 0.05 level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and PChE abnormal level in growing period. There was no significant association between age, education, working hours, working years, smoking and eating/drinking and alcohol drinking at the work , use of PPE & washing hands after working and PChE abnormal level in growing period. It could be found that only p-value < 0.2 level was found in alcohol drinking at the work & washing hands after working and PChE abnormal level in growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including two risk factors namely alcohol drinking at the work & washing hands after working. So multiple logistic regression analysis was done exploring all possible risk factors for alcohol drinking at the work , use of PPE & washing hands after working with PChE abnormal in growing period (p-value were 0.174 and 0.174) respectively were shown in Table 4.29.

Table 4. 29 Multiple logistic regressions of work related factors and PChE in growing period (n = 100)

Plasma cholinesterase (PChE) (Growing period)			
Variables	**Multiple logistic regression**		
	Adjusted OR	**95% CI**	**p value**
Alcohol drinking at the work			
No	1	-	
Yes	1.884	0.825-4.3000	0.133
Washing Hands After Working			
Hand washings	1	-	
No Hand washings	0.534	0.236–1.209	0.133

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; ‘p’ significant at $\alpha = 0.05$

According to the study results, alcohol drinking at the work & washing hands after working are not significantly associated with PChE abnormal in growing period. Adjusted the factors, the odd of history of alcohol drinking at the work group participants were 1.88 times more effect to get PChE abnormal than non- alcohol drinking at the work group, the odd of history of no hand washing group participants were 0.53 lesser chance to have abnormal PChE level compared to hand washing group participants at the work in this study.

4.2.6.1.6 Factor associated Plasma cholinesterase: PChE in Non-Growing Period

Factor associated between plasma cholinesterase monitoring (PChE) in non-growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in non-growing period by bivariate logistic regression and shows in Table 4.30.

Table 4. 30 Bivariate analysis of work related factors and PChE abnormal in non-growing period (n = 100)

Plasma Cholinesterase (PChE) (Non-growing Period)					
Variables	**Normal PChE**	**Abnormal PChE**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	19(19%)	33(33%)	1	-	
≥ 40 years old	31(31%)	17(17%)	0.316	0.139-0.715	0.005*
Education					
< middle school level	14(14%)	20(20%)	1	-	
≥middle school level	36(36%)	30(30%)	0.583	0.252-1.348	0.205
Working Hours					
<5 hours	13(13%)	13(13%)	1	-	
≥5 hours	37(37%)	37(37%)	1.000	0.409–2.444	1.000
Working Years					
<10 years	8(8%)	7(7%)	1	-	
≥10 years	42(42%)	43(43%)	1.170	0.390–3.515	0.779
Smoking at the work					
No	20(20%)	18(18%)	1	-	
Yes	30(30%)	32(32%)	1.185	0.528-2.660	0.680
Eating/drinking at the work					
No	11(11%)	13(13%)	1	-	
Yes	39(39%)	37(37%)	0.803	0.320–2.015	0.640
Alcohol drinking at the work					
No	25(25%)	30(30%)	1	-	
Yes	25(25%)	20(20%)	0.667	0.302–1.472	0.315

Use of PPE					
Use of PPE	5(5%)	6(6%)	1	-	
Non-use of PPE	45(45%)	44(44%)	0.815	0.232-2.865	0.749
Washing Hands After Working					
Hand washings	24(24%)	28(28%)	1	-	
No Hand washings	26(26%)	22(22%)	0.725	0.330–1.594	0.423

Using X^2 test; ‘p’ significant at $\alpha = 0.05$ level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and PChE abnormal level in non-growing period. There is a statistically significant association between age (p-value<0.05) and PChE abnormal level in non-growing period. It could be found that only p-value < 0.2 level was found only in age group and PChE abnormal level in non-growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, working hours, working years, smoking and eating/drinking and alcohol drinking at the work use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including only one risk factor namely age group. So multiple logistic regression analysis was done exploring all possible risk factors for age group with PChE abnormal in non-growing period (p-value were 0.005) was shown in Table 4.31.

Table 4. 31 Multiple logistic regressions of work related factors and PChE abnormal in non-growing period (n = 100)

Plasma cholinesterase (PChE) (Non-growing period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Age			
< 40 years old	1	-	
≥ 40 years old	0.316	0.139–0.715	0.006*

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; 'p' significant at α = 0.05

According to the study results, age group are statistically significantly associated with PChE abnormal in non-growing period (p-value <0.05). Adjusted the factor, the odd of older age group were 0.32 times lesser chance to get PChE abnormal than younger age group participants among non-growing period in this study.

4.2.6.2 Factor associated between Blood Hormonal Assay Monitoring and Work Related Factors in Growing Period and Non-Growing Period

4.2.6.2.1 Factor associated Follicle-stimulating hormone (FSH) in Growing Period

Factor associated between Follicle-stimulating hormone (FSH) in growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in non-growing period by bivariate analysis and shows in Table 4.32.

Table 4. 32 Bivariate analysis of work related factors and FSH abnormal in growing period (n = 100)

Follicle-stimulating hormone (FSH) (Growing Period)					
Variables	**Normal FSH**	**Abnormal FSH**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	49(49%)	3(3%)	1	-	
≥ 40 years old	43(43%)	5(5%)	1.899	0.429-8.417	0.392
Education					
< middle school level	31(31%)	3(3%)	1	-	
≥middle school level	61(61%)	5(5%)	0.847	0.190-3.778	0.828
Working Hours					
<5 hours	21(21%)	5(5%)	1	-	
≥5 hours	71(71%)	3(3%)	0.177	0.039–0.805	0.014
Working Years					
<10 years	14(14%)	1(1%)	1	-	
≥10 years	78(78%)	7(7%)	1.256	0.143–11.02	0.836
Smoking at the work					
No	33(33%)	5(5%)	1	-	
Yes	59(59%)	3(3%)	0.336	0.075-1.494	0.137
Eating/drinking at the work					
No	23(23%)	1(1%)	1	-	
Yes	69(69%)	7(7%)	2.333	0.272–19.99	0.427
Alcohol drinking at the work					
No	51(51%)	4(4%)	1	-	
Yes	41(41%)	4(4%)	1.244	0.293–5.280	0.767
Use of PPE					

Use of PPE	11(11%)	0(0%)	1	-	
Non-use of PPE	81(81%)	8(8%)	1.099	1.029-1.173	0.300
Washing Hands After Working					
Hand washings	49(49%)	3(3%)	1	-	
No Hand washings	43(43%)	5(5%)	1.899	0.429–8.417	0.392

Using X^2 test; 'p' significant at $\alpha = 0.05$ level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and FSH abnormal level in growing period. There was statistically significant association between working hours and FSH abnormal level in growing period (p-value<0.05). It could be found that only p-value < 0.2 level was found in working hours & smoking at the work and FSH abnormal level in growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including two risk factors namely working hours & smoking at the work. So multiple logistic regression analysis was done exploring all possible risk factors for working hours & smoking at the work with FSH abnormal in growing period (p-value were 0.014 and 0.137) respectively were shown in Table 4.33.

Table 4. 33 Multiple logistic regressions of work related factors on FSH in growing period (n = 100)

Follicle-stimulating hormone (FSH) (Growing Period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Working Hours			
<5 hours	1	-	
≥5 hours	0.170	0.036–0.791	0.024*
Smoking at the work			
No	1	-	
Yes	0.315	0.067–1.479	0.143

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; ‘p’ significant at $\alpha = 0.05$

According to the study results, working hours are statistically significantly associated with FSH abnormal in growing period (p-value <0.05). Adjusted the factors, the odd of working hours more than 5 hours were 0.17 times lesser chance to have abnormal FSH level compared to lesser working hours and the odd of smoking at the work group were 0.31 times less chance to get abnormal FSH level than no smoking at the work group in this study.

4.2.6.2.2 Factor associated Follicle-stimulating hormone (FSH) in Non-Growing Period

Factor associated between Follicle-stimulating hormone (FSH) in non-growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in non-growing period by bivariate analysis and shows in Table 4.34.

Table 4. 34 Bivariate analysis of work related factors on FSH in non-growing period (n = 100)

Follicle-stimulating hormone (FSH) (Non-growing Period)					
Variables	**Normal FSH**	**Abnormal FSH**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	49(49%)	3(3%)	1	-	
≥ 40 years old	41(41%)	7(7%)	2.789	0.678-11.48	0.142
Education					
< middle school level	31(31%)	3(3%)	1	-	
≥middle school level	59(59%)	7(7%)	1.226	0.296-5.075	0.778
Working Hours					
<5 hours	22(22%)	4(4%)	1	-	
≥5 hours	68(68%)	6(6%)	0.485	0.125–1.878	0.287
Working Years					
<10 years	14(14%)	1(1%)	1	-	
≥10 years	76(76%)	9(9%)	1.658	0.194–14.14	0.641
Smoking at the work					
No	34(34%)	4(4%)	1	-	
Yes	56(56%)	6(6%)	0.911	0.240-3.461	0.891
Eating/drinking at the work					
No	23(23%)	1(1%)	1	-	
Yes	67(67%)	9(9%)	3.090	0.371–25.73	0.275
Alcohol drinking at the work					
No	49(49%)	6(6%)	1	-	
Yes	41(41%)	4(4%)	0.797	0.210–3.017	0.738

Use of PPE					
Use of PPE	10(10%)	1(1%)	1	-	
Non-use of PPE	80(80%)	9(9%)	1.125	0.129-9.834	0.915
Washing Hands After Working					
Hand washings	48(48%)	4(4%)	1	-	
No Hand washings	42(42%)	6(6%)	1.714	0.453–6.490	0.423

Using X^2 test; 'p' significant at $\alpha = 0.05$ level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and FSH abnormal level in non-growing period. There is no significant association between age, education, working hours, working years, smoking and eating/drinking and alcohol drinking at the work, use of PPE & washing hands after working and FSH abnormal level in non-growing period. It could be found that only p-value < 0.2 level was found only in age group and FSH abnormal level in non-growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, working hours, working years, smoking and eating/drinking and alcohol drinking at the work use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including only one risk factor namely age group. So multiple logistic regression analysis was done exploring all possible risk factors for age group with FSH abnormal in non-growing period (p-value were 0.005) was shown in Table 4.35.

Table 4. 35 Multiple logistic regressions of work related factors and FSH abnormal in non-growing period (n = 100)

Follicle-stimulating hormone (FSH) (Non-growing period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Age			
< 40 years old	1	-	
≥ 40 years old	2.789	0.678–11.476	0.155

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; 'p' significant at α = 0.05

According to the study results, age group is not statistically significantly associated with FSH abnormal in non-growing period. Adjusted the factor, the odd of older age group were 2.79 times lesser chance to get FSH abnormal than younger age group participants among non-growing period in this study.

4.2.6.2.3 Factor associated Luteinizing Hormone (LH) in Growing Period

Factor associated between Luteinizing Hormone (LH) in growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in growing period by bivariate analysis and shows in Table 4.36.

Table 4. 36 Bivariate analysis of work related factors on LH abnormal in growing period (n = 100)

Luteinizing Hormone (LH) (Growing Period)					
Variables	**Normal LH**	**Abnormal LH**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	43(43%)	9(9%)	1	-	
≥ 40 years old	25(25%)	23(23%)	4.396	1.761-10.97	0.001*
Education					
< middle school level	23(23%)	11(11%)	1	-	
≥middle school level	45(45%)	21(21%)	0.976	0.402-2.366	0.957
Working Hours					
<5 hours	15(15%)	11(11%)	1	-	
≥5 hours	53(53%)	21(21%)	0.540	0.214–1.366	0.190
Working Years					
<10 years	11(11%)	4(4%)	1	-	
≥10 years	57(57%)	28(28%)	1.351	0.395–4.624	0.631
Smoking at the work					
No	27(27%)	11(11%)	1	-	
Yes	41(41%)	21(21%)	1.257	0.523-3.020	0.608
Eating/drinking at the work					
No	20(20%)	4(4%)	1	-	
Yes	48(48%)	28(28%)	2.917	0.905–9.401	0.065
Alcohol drinking at the work					
No	35(35%)	20(20%)	1	-	
Yes	33(33%)	12(12%)	0.636	0.269–1.503	0.301

Use of PPE					
Use of PPE	9(9%)	2(2%)	1	-	
Non-use of PPE	59(59%)	30(30%)	2.288	0.465-11.27	0.298
Washing Hands After Working					
Hand washings	41(41%)	11(11%)	1	-	
No Hand washings	27(27%)	21(21%)	2.899	1.207–6.964	0.016*

Using X^2 test; ‘p’ significant at α = 0.05 level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and LH abnormal level in growing period. There was statistically significant association between age group & washing hands after working and LH abnormal level in growing period (p-value<0.05). It could be found that only p-value < 0.2 level was found in age group, working hours, eating and drinking at the work & washing hands after the work and LH abnormal level in growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including four risk factors namely age group, working hours, eating and drinking at the work & washing hands after the work. So multiple logistic regression analysis was done exploring all possible risk factors for age group, working hours, eating and drinking at the work & washing hands after the work with LH abnormal in growing period (p-value were 0.001,0.190, 0.065 and 0.016) respectively were shown in Table 4.37.

Table 4. 37 Multiple logistic regressions of work related factors and LH abnormal in growing period (n = 100)

Luteinizing Hormone (LH) (Growing Period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Age			
< 40 years old	1	-	
≥ 40 years old	4.098	1.574–10.667	0.004*
Working Hours			
<5 hours	1	-	
≥5 hours	0.632	0.219–1.824	0.396
Eating/drinking at the work			
No	1	-	
Yes	2.903	0.844–9.990	0.091
Washing Hands After Working			
Hand washings	1	-	
No Hand washings	2.529	0.969–6.598	0.058

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; ‘p’ significant at $\alpha = 0.05$

According to the study results, age group is statistically significantly associated with LH abnormal in growing period (p-value<0.05). Adjusted the factor, the odd of history of older age group were 4.09 times more chance to get LH abnormal than the younger age group, the odd of above 5 hours working group participants were 0.63 times less chance to have abnormal LH level compared to less than 5 hours working group , the odd of eating and drinking at the work group participants were 2.90 times more getting chance to become LH abnormal than not eating and drinking group participants and the odd of having washing hands after the work group participants were 2.53 times more chance to get LH abnormal compared to no history of washing hands after the work group participants in this study.

4.2.6.2.4 Factor associated Luteinizing Hormone (LH) in Non-Growing Period

Factor associated between Luteinizing Hormone (LH) in non-growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in non-growing period by bivariate analysis and shows in Table 4.38.

Table 4. 38 Univariate analysis of work related factors on LH in non-growing period (n = 100)

Luteinizing Hormone (LH) (Non-growing Period)					
Variables	**Normal LH**	**Abnormal LH**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	43(43%)	9(9%)	1	-	
≥ 40 years old	32(32%)	16(16%)	2.389	0.937-6.092	0.064
Education					
< middle school level	27(27%)	7(7%)	1	-	
≥middle school level	48(48%)	18(18%)	1.446	0.536-3.901	0.465
Working Hours					
<5 hours	20(20%)	6(6%)	1	-	
≥5 hours	55(53%)	19(19%)	1.152	0.403–3.294	0.792
Working Years					
<10 years	12(12%)	3(3%)	1	-	
≥10 years	63(63%)	22(22%)	1.397	0.360–5.415	0.628
Smoking at the work					
No	31(31%)	7(7%)	1	-	
Yes	44(44%)	18(18%)	1.812	0.675-4.859	0.234

Eating/drinking at the work					
No	22(22%)	2(2%)	1	-	
Yes	53(53%)	23(23%)	4.774	1.036–22.00	0.031*
Alcohol drinking at the work					
No	41(41%)	14(14%)	1	-	
Yes	34(34%)	11(11%)	0.947	0.381–2.357	0.908
Use of PPE					
Use of PPE	10(10%)	1(1%)	1	-	
Non-use of PPE	65(59%)	24(24%)	3.692	0.448-30.40	0.196
Washing Hands After Working					
Hand washings	40(40%)	12(12%)	1	-	
No Hand washings	35(35%)	13(13%)	1.238	0.500–3.065	0.644

Using X^2 test; ‘p’ significant at α = 0.05 level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and LH abnormal level in non-growing period. There was statistically significant association between eating and drinking at the work and LH abnormal level in non-growing period (p-value<0.05). It could be found that only p-value < 0.2 level was found in age group, eating and drinking at the work & use of PPE and LH abnormal level in non-growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including three risk factors namely age group, eating and drinking at the work & use of PPE. So multiple logistic regression analysis was done exploring all possible risk factors for age group, eating and drinking at the work & use of PPE with

LH abnormal in non-growing period (p-value was 0.064,0.031,0.196) respectively were shown in Table 4.39.

Table 4. 39 Multiple logistic regressions of work related factors on LH in non-growing period (n = 100)

Luteinizing Hormone (LH) (Non-growing Period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Age			
< 40 years old	1	-	
≥ 40 years old	2.390	0.906–6.304	0.078
Eating/drinking at the work			
No	1	-	
Yes	5.030	1.067–23.714	0.041*
Use of PPE			
Use of PPE	1	-	
Non-use of PPE	4.438	0.516–38.163	0.175

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; 'p' significant at $\alpha = 0.05$

According to the study results, eating and drinking at the work is statistically significantly associated with LH abnormal in non-growing period (p-value<0.05). Adjusted the factor, the odd of history of older age group were 2.39 times more chance to get LH abnormal than the younger age group, the odd of eating/ drinking group participants were 5.03 times more chance to have abnormal LH level compared to no history of eating and drinking group participants at the work and the odd of having not use of PPE group participants were 4.44 times more chance to get LH abnormal compared to use of PPE group participants in this study.

4.2.6.2.5 Factor associated Testosterone in Growing Period

Factor associated between Testosterone in growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in non-growing period by bivariate analysis and shows in Table 4.40.

Table 4. 40 Bivariate analysis of work related factors and Testosterone abnormal in growing period (n = 100)

Testosterone (Growing Period)					
Variables	**Normal Testoste-rone**	**Abnormal Testoste-rone**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	48(48%)	4(4%)	1	-	
≥ 40 years old	40(40%)	8(8%)	2.400	0.673-8.559	0.168
Education					
< middle school level	30(30%)	4(4%)	1	-	
≥middle school level	58(58%)	8(8%)	1.034	0.288-3.715	0.959
Working Hours					
<5 hours	23(23%)	3(3%)	1	-	
≥5 hours	65(65%)	9(9%)	1.062	0.264–4.264	0.933
Working Years					
<10 years	13(13%)	2(2%)	1	-	
≥10 years	75(75%)	10(10%)	0.867	0.170–4.416	0.863
Smoking at the work					
No	30(30%)	8(8%)	1	-	
Yes	58(58%)	4(4%)	0.259	0.072-0.929	0.029*
Eating/drinking at the work					
No	21(21%)	3(3%)	1	-	
Yes	67(67%)	9(9%)	0.940	0.233–3.796	0.931
Alcohol drinking at the work					
No	50(50%)	5(5%)	1	-	
Yes	38(38%)	7(7%)	1.842	0.542–6.256	0.322

Use of PPE					
Use of PPE	10(10%)	1(1%)	1	-	
Non-use of PPE	78(78%)	11(11%)	1.410	0.164-12.11	0.753
Washing Hands After Working					
Hand washings	45(45%)	7(7%)	1	-	
No Hand washings	43(43%)	5(5%)	0.748	0.220–2.535	0.640

Using X^2 test; 'p' significant at α = 0.05 level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and Testosterone abnormal level in growing period. There was statistically significant association between smoking at the work and Testosterone abnormal level in growing period (p-value<0.05). It could be found that only p-value < 0.2 level was found in age group & smoking at the work and Testosterone abnormal level in growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including two risk factors namely age group & smoking at the work. So multiple logistic regression analysis was done exploring all possible risk factors for age group & smoking at the work with Testosterone abnormal in growing period (p-value were 0.168 and 0.029) respectively were shown in Table 4.41.

Table 4. 41 Multiple logistic regressions of work related factors on Testosterone in growing period (n = 100)

Testosterone (Growing Period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Age			
< 40 years old	1	-	
≥ 40 years old	2.547	0.691–9.388	0.160
Smoking at the work			
No	1	-	
Yes	0.248	0.068–0.907	0.035*

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; ‘p’ significant at α = 0.05

According to the study results, smoking at the work is statistically significantly associated with Testosterone abnormal in growing period (p-value<0.05). Adjusted the factor, the odd of older age group were 2.55 times more chance to get Testosterone abnormal than younger age group and the odd of smoking at the work group participants were 0.25 times lesser chance to have abnormal Testosterone level compared to no history of smoking at the work group participants in this study.

4.2.6.2.6 Factor associated Testosterone in Non-Growing Period

Factor associated between Testosterone in non-growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in non-growing period by bivariate analysis and shows in Table 4.42.

Table 4. 42 Bivariate analysis of work related factors and Testosterone abnormal in non-growing period (n = 100)

Testosterone (Non-growing Period)					
Variables	**Normal Testoste-rone**	**Abnormal Testoste-rone**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	46(46%)	6(6%)	1	-	
≥ 40 years old	36(36%)	12(12%)	2.556	0.874-7.470	0.080
Education					
< middle school level	24(24%)	10(10%)	1	-	
≥middle school level	58(58%)	8(8%)	0.331	0.117-0.941	0.033*
Working Hours					
<5 hours	21(21%)	5(5%)	1	-	
≥5 hours	61(61%)	13(13%)	0.895	0.285–2.811	0.849
Working Years					
<10 years	14(14%)	1(1%)	1	-	
≥10 years	68(68%)	17(17%)	3.500	0.430–28.50	0.215
Smoking at the work					
No	31(31%)	7(7%)	1	-	
Yes	51(51%)	11(11%)	0.955	0.335-2.723	0.932
Eating/drinking at the work					
No	22(22%)	2(2%)	1	-	
Yes	60(60%)	16(16%)	2.933	0.623–13.81	0.157
Alcohol drinking at the work					
No	43(43%)	12(12%)	1	-	
Yes	39(39%)	6(6%)	0.551	0.189–1.610	0.272

Use of PPE					
Use of PPE	10(10%)	1(1%)	1	-	
Non-use of PPE	72(72%)	17(17%)	2.361	0.283-19.72	0.415
Washing Hands After Working					
Hand washings	44(44%)	8(8%)	1	-	
No Hand washings	38(38%)	10(10%)	1.447	0.519–4.038	0.479

Using X^2 test; 'p' significant at $\alpha = 0.05$ level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and Testosterone abnormal level in non-growing period. There was statistically significant association between education and Testosterone abnormal level in non-growing period (p-value<0.05). It could be found that only p-value < 0.2 level was found in age group, education & eating/drinking at the work and Testosterone abnormal level in non-growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including three risk factors namely age group, education & eating/drinking at the work. So multiple logistic regression analysis was done exploring all possible risk factors for age group & smoking at the work with Testosterone abnormal in non-growing period (p-value were 0.080, 0.033 and 0.057) respectively were shown in Table 4.43.

Table 4. 43 Multiple logistic regressions of work related factors and Testosterone abnormal in non-growing period (n = 100)

Testosterone (Non-growing Period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Age			
< 40 years old	1	-	
≥ 40 years old	2.464	0.814–7.459	0.111
Education			
< middle school level	1	-	
≥middle school level	0.325	0.111–0.954	0.041*
Eating/drinking at the work			
No	1	-	
Yes	2.825	0.578–13.814	0.200

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; 'p' significant at $\alpha = 0.05$

According to the study results, education is statistically significantly associated with Testosterone abnormal in non-growing period (p-value<0.05). Adjusted the factor, the odd of older age group participants were 2.46 times more chance to have abnormal Testosterone level compared to younger age group, the odd of higher education level were 0.33 times lesser to have abnormal Testosterone level compared to lower education level and the odd of eating and drinking at the work age group were 2.83 times more chance to have abnormal Testosterone level than no eating and drinking age group this study.

4.2.6.3 Factor associated between Seminal Assay Monitoring and Work Related Factors in Growing Period and Non-Growing Period

4.2.6.3.1 Factor associated Sperm Count in Growing Period

Factor associated between Sperm Count in growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in growing period by bivariate analysis and shows in Table 4.44.

Table 4. 44 Bivariate analysis of work related factors and Sperm Count abnormal in growing period (n = 100)

Sperm Count (Growing Period)					
Variables	**Normal Sperm Count**	**Abnormal Sperm Count**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	13(13%)	39(39%)	1	-	
≥ 40 years old	10(10%)	38(38%)	1.267	0.496-3.238	0.621
Education					
< middle school level	8(8%)	26(26%)	1	-	
≥middle school level	15(15%)	51(51%)	1.046	0.393-2.786	0.928
Working Hours					
<5 hours	5(5%)	21(21%)	1	-	
≥5 hours	18(18%)	56(56%)	0.741	0.244–2.249	0.595
Working Years					
<10 years	4(4%)	11(11%)	1	-	
≥10 years	19(19%)	66(66%)	1.263	0.361–4.422	0.714
Smoking at the work					
No	10(10%)	28(28%)	1	-	
Yes	13(13%)	49(49%)	1.346	0.523-3.467	0.537
Eating/drinking at the work					
No	2(2%)	22(22%)	1	-	
Yes	21(21%)	55(55%)	0.238	0.051–1.102	0.050

Alcohol drinking at the work					
No	16(16%)	39(39%)	1	-	
Yes	7(7%)	38(38%)	2.227	0.824–6.019	0.110
Use of PPE					
Use of PPE	2(2%)	9(9%)	1	-	
Non-use of PPE	21(21%)	68(68%)	0.720	0.144-3.594	0.687
Washing Hands After Working					
Hand washings	11(11%)	41(41%)	1	-	
No Hand washings	12(12%)	36(36%)	0.805	0.317–2.045	0.648

Using X^2 test; 'p' significant at α = 0.05 level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and Sperm Count abnormal level in growing period. There was no statistically significant association between age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working and Sperm Count abnormal level in growing period. It could be found that only p-value < 0.2 level was found in eating/drinking & alcohol at the work and Sperm Count abnormal level in growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including two risk factors namely eating/drinking & alcohol drinking at the work. So multiple logistic regression analysis was done exploring all possible risk factors for eating/drinking & alcohol drinking at the work with Sperm Count abnormal in growing period (p-value were 0.050 and 0.110) respectively were shown in Table 4.45.

Table 4. 45 Multiple logistic regressions of work related factors and Sperm Count abnormal in growing period (n = 100)

Sperm Count (Growing Period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Eating/drinking at the work			
No	1	-	
Yes	0.233	0.050–1.092	0.065
Alcohol drinking at the work			
No	1	-	
Yes	2.276	0.827–6.264	0.111

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; 'p' significant at $\alpha = 0.05$

According to the study results, there was no statistically significantly associated with Sperm Count abnormal in growing period. Adjusted the factor, the odd of eating/drinking at the work group participants were 0.23 times lesser chance to have abnormal Sperm Count level compared to no history of eating/drinking at the work group participants and the odd of alcohol drinking participants were 2.28 times more chance to suffered decrease sperm count than no history of alcohol drinking participants in this study.

4.2.6.3.2 Factor associated Sperm Count in Non-Growing Period

Factor associated between Sperm Count in non-growing period with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working in non-growing period by bivariate analysis and shows in Table 4.46.

Table 4. 46 Bivariate analysis of work related factors on Sperm Count in non-growing period (n = 100)

Sperm Count (Non-growing Period)					
Variables	**Normal Sperm Count**	**Abnormal Sperm Count**	**Bivariate analysis**		
			OR	**95% CI**	**p value**
Age					
< 40 years old	30(30%)	22(22%)	1	-	
≥ 40 years old	24(24%)	24(24%)	1.364	0.619-3.002	0.441
Education					
< middle school level	17(17%)	17(17%)	1	-	
≥middle school level	37(37%)	29(29%)	0.784	0.342-1.797	0.565
Working Hours					
<5 hours	13(13%)	13(13%)	1	-	
≥5 hours	41(18%)	33(33%)	0.805	0.329–1.970	0.634
Working Years					
<10 years	10(10%)	5(5%)	1	-	
≥10 years	44(44%)	41(41%)	1.864	0.587–5.914	0.286
Smoking at the work					
No	23(23%)	15(15%)	1	-	
Yes	31(31%)	31(31%)	1.533	0.676-3.478	0.305
Eating/drinking at the work					
No	14(14%)	10(10%)	1	-	
Yes	40(40%)	36(36%)	1.260	0.498–3.187	0.625
Alcohol drinking at the work					
No	31(31%)	24(24%)	1	-	
Yes	23(23%)	22(22%)	1.236	0.560–2.725	0.600

Use of PPE					
Use of PPE	8(8%)	3(3%)	1	-	
Non-use of PPE	46(46%)	43(43%)	2.493	0.621-10.01	0.187
Washing Hands After Working					
Hand washings	28(28%)	24(24%)	1	-	
No Hand washings	26(26%)	22(22%)	0.987	0.449–2.169	0.974

Using X^2 test; ‘p’ significant at α = 0.05 level; OR = Odds Ratio; 95% CI= 95% Confidence Interval

The above table is presented by association between work related factors and Sperm Count abnormal level in non-growing period. There was no statistically significant association between age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working and Sperm Count abnormal level in non-growing period. It could be found that only p-value < 0.2 level was found only in use of PPE and Sperm Count abnormal level in non-growing period. So, predictive modeling was used by unconditional multiple logistic regressions. First of all, we made the modeling with one explanatory variable to identify strongest predictor among age, education, smoking and eating/drinking, alcohol drinking at the work, working hours, working years, use of PPE & washing hands after working. We reduced the risk factors in the model one by one starting with the weakest predictors. We compared the Log-likelihood results of the complex models and less complex ones by Chi-square tests. Finally, the best predictive model was including only one risk factor namely use of PPE. So multiple logistic regression analysis was done exploring all possible risk factors for use of PPE with Sperm Count abnormal in non-growing period (p-value was 0.187) was shown in Table 4.47.

Table 4. 47 Multiple logistic regressions of work related factors and Sperm Count abnormal in non-growing period (n = 100)

Sperm Count (Non-Growing Period)			
Variables	**Multiple logistic regressions**		
	Adjusted OR	**95% CI**	**p value**
Use of PPE			
Use of PPE	1	-	
Non-use of PPE	2.493	0.621–10.013	0.198

Using unconditional logistic regressions; 95% CI = 95% Confidence interval; 'p' significant at α = 0.05

According to the study results, there was no statistically significantly associated with Sperm Count abnormal in non-growing period. Adjusted the factor, the odd of non-use of PPE group participants were 2.49 times more chance to have abnormal Sperm Count level compared to use of PPE group participants in this study.

4.2.7 Pesticide Residues on Hands

To do dermal exposure assessment and health risk assessment, thirty ground-nut farmers were selected from the Phase II by simple random sampling. Chlorpyrifos were chosen for dermal exposure assessment because in this village, the participants use mainly its chemical for their farms and the residue samples were carefully collected once from each farmer immediately after application of chlorpyrifos. A modified version of Lappharat S studied (139) and P. Ong-artborirak studied (170), was used to assess exposure to pesticide residues (PRs) from patch samples.

In this study for dermal exposure assessment, all of the samples of 30 ground-nut farmers who used the Chlorpyrifos (Organophosphate group) in the growing season were examined and mean ± SD was 257.98 ± 165.41 with the range of 57.18 - 743.30. Individuals concentration of pesticide on hands (mg/2hands) were shown in table 4.48.

Table 4. 48 Concentration of pesticide on hand (µg/2 hands) by wipe test (n = 30)

1. Sample 1 - 743.3	11. Sample 11 – 133	21. Sample 21 – 252.8
2. Sample 2 - 104.2	12. Sample 12 - 111	22. Sample 22 – 296.2
3. Sample 3 – 293.6	13. Sample 13 – 457	23. Sample 23 – 182.2
4. Sample 4 – 84.2	14. Sample 14 – 657.6	24. Sample 24 – 310
5. Sample 5 – 222.2	15. Sample 15 – 476.3	25. Sample 25 – 285.9
6. Sample 6 – 105.8	16. Sample 16 – 247.4	26. Sample 26 – 146.5
7. Sample 7 – 372.7	17. Sample 17 – 122.3	27. Sample 27 – 221.5
8. Sample 8 – 122.8	18. Sample 18 – 353.6	28. Sample 28 – 364.4
9. Sample 9 – 78.71	19. Sample 19 – 115.3	29. Sample 29 – 299.5
10. Sample 10– 57.18	20. Sample 20 – 213.9	30. Sample 30 – 309.2
Mean ± SD = 257.98 ± 165.41 Min-Max = 57.18 - 743.30		

4.2.8 Health Risk Assessment

In the risk assessment, the exposure assessment and risk characterization were a process determining the dose of pesticide exposure and classify the risk of this study population. The definition of Average daily dose (ADD), given by Integrated Risk Information System (IRIS) of US EPA, was “the mean amount of an agent to which a person is exposed on a daily basis, often averaged over a long period of time” (171). The hazard quotient (HQ) and hazard index (HI) were used in the step of risk characterization for showing the ratio of estimated site-exposure to a single chemical from a site over a specific period to the estimated daily exposure level (172). Firstly, body surface area (SA) of study population was calculated which was specific for people in this area. The value of SA was a part of factor to account average daily dose (ADD). The model of DuBois (1996) cited in US EPA 2011 (173) surface area calculation was used in this study (equation 4-1) and age, personal weights, heights and work practice distribution of the 30 respondents were factor for this calculation in Table 4.49.

$SA = a0\ W^{a1}\ H^{a2}$ *eq. 4-1*
Where:

SA = surface area (m^2)

H = height (cm)

W = weight (kg)

a0, a1, a2 = constant values from US EPA, 1997 (166)

The a0, a1, and a2 in this equation were referred to the US EPA's defaults values that were shown in the Appendix F.

Table 4. 49 Age ,weight (kg), height (cm) and work practice distribution. (n=30)

Factors	Median	Range	Mean±SD	Percentile			
				25th	50th	75th	95th
Age (years)	38	18 – 49	37.83±7.49	32.75	38.00	43.25	48.45
Body Weight (kg)	56.69	49.89 -70.30	57.72±5.89	54.43	56.69	61.23	48.45
Body Height (cm)	167.64	152.4-182.9	165.44±6.53	161.29	167.64	170.18	175.89
Exposure duration (years)	19.5	3 – 33	19.2±7.73	13.5	19.5	25	32.45
Working days/week	6	5 – 7	6.27±0.55	6.00	6.00	6.63	7.00
Working hours/day	5	3 – 6	4.97±0.96	4.00	5.00	6.00	6.00
Working days/years	96	80 – 112	100.27±8.85	96	96	106	112

Defaults value of SA calculation

[a] a0 = 0.0257, a1 = 0.573, a2 = -0.218 (US EPA, 1985 cited in US EPA, 1997) for hands

By Calculation :

$SA = a0\ W^{a1}\ H^{a2}$

$= 0.0257\ W^{0.573}\ H^{-0.218}$

$= 0.0257 * 57.72^{0.573} * 165.44^{-0.218}$

$= 0.086\ m^2$

$= 0.86 \times 10^3\ cm^2$ (Average surface area cm² for hands) (166)

Refer to patch samples observation that was beneficial to estimate pesticide residues deposited on ground-nut growing farmers' hands it was calculated the average daily dose. The table 4.46 pointed out the mean, median, minimum, maximum and 95th percentile of chlorpyrifos concentration on hands of male ground-nut farmers. And the reasonable maximum exposure (RME) at 95th percentile was estimated to prevention and protection of high dermal exposure male ground-nut farmers. For calculation on ADD dermal, chlorpyrifos concentration on hands (µg/2hands) of male ground-nut farmers change to (mg/2hands) and shows as the percentile level each at the following table 4.50.

Table 4. 50 Chlorpyrifos concentration on hands (mg/2hands)of male ground-nut farmers (n= 30)

Part of body	**Concentration (mg/2hands)**							
	Mean ±SD	**Median**	**Min:**	**Max:**	**25th**	**50th**	**75th**	**95th**
Hands	0.257±0.165	0.234	0.57	0.743	0.120	0.234	0.321	0.696

The exposure assessment of body skin contact was calculated by the following equation (*eq. 4-2*). This equation was same as dermal average daily dose equation (*eq. 3- 1*).

For skin contact ,

{PR concentration/SA × absorption fraction (ABS)}

$$\text{ADD dermal (mg/kg} - \text{day)} \ = \frac{\text{DA event} \times \text{EV} \times \text{ED} \times \text{EF} \times \text{SA}}{\text{BW} \times \text{AT}}$$

ADD = average daily dose (mg/kg-day)

DA event = absorbed dose per event (mg/ cm2-event)

PR concentration = At 95th percentile level (mg/2hnads)(US EPA, 1992) (153)

Absorption fraction(ABS) = 0.03 for chlorpyrifos From US EPA, 2002 (116)

EV = event frequency (events/day) (1 event/day)

ED = exposure duration (years) (19.2 years from questionnaire)

EF = exposure frequency (days/years) (6.27 days/16 weeks/year from questionnaire)

SA = skin surface area available for contact (cm2) (**0.86×10^3 cm^2**) (166)

BW = body weight (kg) (57.72 kg from questionnaire)

AT = average time (days) for non-carcinogenic effects

(19.2 years × 365 days/year)

$$\text{Simplified to, DAevent} \ = \frac{\text{Cs mg of pesticide}}{\text{Kg of gauze weight}} \times \frac{\text{Weight (kg)}}{\text{1 gauze}} \times \frac{\text{1 gauze}}{\text{SA (cm}^2\text{event)}} \times \text{ABS}$$

For Calculation,

$$\text{ADD dermal (mg/kg} - \text{day)} \ = \frac{\text{DA event} \times \text{EV} \times \text{ED} \times \text{EF} \times \text{SA}}{\text{BW} \times \text{AT}}$$

(*eq. 4-2*)

$$= \frac{\frac{\text{Cs mg of pesticide}}{\cancel{\text{Kg of gauze weight}}} \times \frac{\cancel{\text{Weight (kg)}}}{\cancel{\text{1 gauze}}} \times \frac{\cancel{\text{1 gauze}}}{\cancel{\text{SA (cm}^2\text{event)}}} \times \text{ABS} \times \frac{\mathit{event}}{\cancel{\text{day}}} \times \cancel{\text{year}} \times \frac{\cancel{\text{day}}}{\cancel{\text{year}}} \times \cancel{\text{SA}}}{\text{kg} \times \text{day}}$$

$$\mathrm{ADD(mean)} = \frac{\{0.257/860 \times 0.03\} \times 1 \times 19.2 \times 100.27 \times 860}{57.72 \times 7008}$$

= 0.0000366 mg/kg-day

= 3.66 $\times 10^{-5}$ mg/kg-day

$$\mathrm{ADD(RME)} = \frac{\{0.696/860 \times 0.03\} \times 1 \times 19.2 \times 100.27 \times 860}{57.72 \times 7008}$$

= 0.0000993 mg/kg-day

= 9.93$\times 10^{-5}$ mg/kg-day

The value at 95th percentile level of detected pesticide residues concentration on hands (high-end exposure) was used to examine dermal exposure among ground-nut farmers based on fluctuation of amounts. The 95th percentile of the mean concentration is applied as the average concentration, because it is not possible to know the true mean. Due to the less limited sampling data at a site, uncertainties decrease so that the upper confident level moves closer to the true mean. As a consequence, the exposure evaluations using either the mean or the upper confident level produce similar results (153).

The two types of exposure estimates now as required for risk assessments, a reasonable maximum exposure (RME) and an average, should both use average concentration. The RME, which is defined as the highest exposure that could reasonably be expected to occur for a given exposure pathway at a site, is intended to account for both uncertainty in the contaminant concentration and variability in exposure parameters. (e.g., exposure frequency, averaging time). It was states that an average estimate of exposure also should be presented in risk assessments (153).

Exposure assessment revealed the average daily dose ADD (mean) of ground-nut farmers at 3.66 $\times 10^{-5}$ mg/kg-day and ADD (RME) as 9.93 $\times 10^{-5}$ mg/kg-day in growing period among randomly selected 30 samples of ground-nut farmers. For non-carcinogenic risk characterization, Hazard Quotient (HQ) was applied to estimate risk and calculated by the following equation, which provided from US EPA (1997) (166).

$$\text{Hazard Quotient (HQ)} = \frac{\text{Exposure}}{\text{RfD}} \qquad \text{eq. } 4-3$$

Where:

Exposure = chemical exposure level (mg/kg/day)

RfD = reference dose (mg/kg/day)

The description of HQ was presented beneath:

HQ > 1 adverse non-carcinogenic effect concern

HQ ≤1 acceptable level (no concern)

The ADD value from above equation 4-2 was represented in exposure factor (in *eq. 4-3)* and the reference dose was specific for chemical, chlorpyrifos through dermal contact was 0.0003 from US EPA, 2002 (116).

By Calculation,

$$\text{Hazard Quotient (HQ)} = \frac{\text{Exposure}}{\text{RfD}}$$

$$\text{HQ(mean)} = \frac{0.0000366}{0.0003}$$

= 0.12

$$\text{HQ(RME)} = \frac{0.0000993}{0.0003}$$

= 0.33

Risk characterization was the last step of human health risk assessment. It represents both qualitative and quantitative data, which is a tool to link with the risk manager or decision makers. The risk characterization is a process that merges and uses the appropriate method to analyze the necessary information from the hazard identification, dose response assessment, and exposure assessment to make risk estimates for the exposure of interest. OPs are regarded as non-carcinogenic pesticide. The criterion, that is the reference dose (RfD) is used as non-carcinogen risk characterization. The individual risks evaluation of non-carcinogenic toxicity is calculated applying the hazard quotient (HQ) ratio that points out the degree of exposure, greater or less than the RfD. If the exposure is greater than the RfD, the exposure population may be in danger (158)

The choice of the arithmetic mean concentration as a relevant measure to estimate exposure derived from the need to estimate an individual's long-term average exposure. Most Agency health criteria are based on the long-term average daily dose, which is simply the sum of all daily doses divided by the total number of days in the averaging period. This is the definition of arithmetic mean. The arithmetic mean is suitable regardless of the pattern of daily exposures over time, or the type of statistical distribution that might best describe the sampling data. The geometric mean of a set of sampling results, however, expresses no logical connection to the cumulative intake that would result from long-term contact with the site contaminations, and it may differ appreciably from and be much lower than the arithmetic mean. Although the geometric mean is a convenient parameter for describing central tendencies of lognormal distributions, it is not a suitable basis to estimate the concentration term used in Superfund exposure assessments. An estimated of average concentration is used because; (1) carcinogenic and chronic non-carcinogenic toxicity criteria are based on lifetime average exposures and (2) average concentration is most representative of the concentration that would be contacted at a site, over time (174).

In this study, the individual pesticide exposure parameters were calculated using arithmetic mean and upper bound of the 95th percentile. The reasonable maximum exposure (RME) was applied here as the highest exposure that was expected to occur at a study site. The intent of the RME at the upper confidence limit (the 95th percentile) was to estimate this conservative exposure case that was still within the range of possible exposures.

The results in this study showed that dermal exposure to chlorpyrifos residue on hands exposure of male ground-nut farmers in this village were (ADD=3.66 $\times 10^{-5}$) and (HQ=0.12). If HQ value exceeded more than "1", the farming population should be made aware of the health effect through dermal exposure and correct pesticide usage and training should be offered to farmers but in this study, HQ value was less than "1", we can conclude that there is no concern for potential non-carcinogenic effect from dermal exposure to pesticides among ground-nut farmers in the Kyauk-Kan Village of Nyaung-U District, Mandalay Region, Myanmar.

CHAPTER V
DISCUSSION

Being a developing agricultural country, Myanmar have to inevitably use pesticides in agricultural food production. Pesticides are human made or naturally occurring chemicals that control insects, weeds, fungi, and other pest that destroy crops. Knowing how to react to pesticide poisoning is essential. Pesticide can cause adverse health outcomes for human health and animals. In this study involved three parts, named as phase I, II, and III. The first part, phase I was cross-sectional study named as observational study, identified the health problems related to pesticide exposure, explored knowledge and practice on safe use of pesticide among ground-nut farmers among both male and female between May 14 to 31, 2016 in the community by doing face to face interview. For the second and third part, phase II and III were also a cross-sectional study and named as laboratory study, found out the effect of chemicals on male reproductive function of ground-nut farmers who were chronically exposed to organophosphate pesticides by using biomarkers in two periods: 1st time growing period June 8 to 18, 2016 And 2nd time non-growing period at December 17 to 27, 2016. For phase III, hand wipe test was done in growing period.

5.1 General Information of Participants for Observational Study (Phase I)

The phase I, observational study was done in Kyauk Kan village of Nyaung-U District, Mandalay Region, Myanmar. All the respondents were ground-nut farmers who grow ground-nut as their traditional jobs. Nearly one-third of the respondents were in 38 to 47 years age group. These finding are similar to Recena et al., 2006 that indicated that 26.0% of the participants were between the ages group of 35 years to 45 years (175). In this study, about two-third of the respondents was male so the number of males was greater than females. Similarly, the number of males was greater than females in Myo Min, 2006 (16)and Khin Maung Nyunt, 2015 studies (28). In Myanmar, most of the farm works were usually done by male workers.

Two third of the respondents in this study (43.8%) had primary education level and only 8.5% had university and graduate level education, which was in accordance with a study conducted in Khin Maung Nyunt, 2015 in Myanmar (28). Therefore, there is not much difference in the demographic characteristics of the two studies. More lack of education lead to more dose of pesticide exposure. Elementary education was the major group of population in this study. Safety instructions on containers are often written in unfamiliar languages, many farmers are illiterate, and the instructions themselves are difficult to follow (176). Even though, they had a higher education but most of them still used over amount of pesticide instruction. Moreover, they mixed a variety kind of pesticides together in one time. Most farmers gain approximately 1000001- 1500000 kyats (1 USD = 1000 kyats) per year depending on their cultivation area and whether they own the land or not. However, some farmers were hired as pesticide sprayer and were hourly paid. Present observational study shows that 28.2% of farmers were smoker. The results were supported by other study showing that about 40% in Wilaiwan's study (168) and 34% in Yassin's study (117) among the farm workers were smokers.

Nearly half of the respondents had 10-12 years work duration. Average duration of the work was 10 years which mean that these farm workers had used with pesticide for ten years which is similar to a previous studies of Catano's study (177) and Wilaiwan's study (168), respectively. The number of years of using pesticide was higher because ground-nut growing is a major work in the area. Finishing primary school, they started working in ground-nut industry right way.

Most of the farmers applied pesticides to their fields about 5 hours per day in the paddy fields and this information indicated that the farmers who work in paddy field could be exposed to pesticides. Most of the farmers who mixed, loaded, and sprayed pesticides by themselves was 51.2% which is similar to Wilaiwan's study (168).

5.1.1 Knowledge and Practice of farm workers on pesticide

In this study, knowledge of pesticide was explored. The average knowledge score of them was 19.81±5.47 with the range of 4 to 33. The majority of them had moderate level of knowledge (53.5%). In knowledge about "route of entry of pesticide into the human body", all of the respondents (100%) know that pesticide can enter the human body through ingestion, inhalation and skin. In the Myanmar studies done by Nwe-Nwe-Oo, 97% knew the oral route, 94% knew the route of entry through the lungs by inhalation and 85% knew the dermal route (129). As compared to the study done by Myo-Min, 96% knew the routes of entry of pesticide into human body (16). So, knowledge about “route of entry of pesticide into the human body” in this study is not different from the other studies. If farm workers have knowledge about route of entry of pesticide, they will protect their body during their utilization of pesticide.

According to this study, most know the clinical features and outcome of acute toxicity of pesticide. The majority (82.8%) has knowledge about acute toxicity of pesticides and (90.8%) have knowledge about the chronic toxicity. This finding was higher than the other studies done by Pyae Phyo Thar in 2012 which found that about 80% knew the adverse health effect of pesticides on human (12). Although the answers were quite satisfactory, all of the farm workers must know about its effect on health and how dangerous it is for environment.

Respondents in this study know synthetic organophosphate and pyrethroid most. In this study, the ground-nut farmers do not use carbamate group. In fact, organophosphate and pyrethroid cause lowest toxicity to human and environment. Choice of these pesticides among the farmers does not depend on their knowledge about pesticides but it may be due to the nature of pests on their farms and availability of this type of pesticide in market. Pyrethroids have two advantages of high biological activity against insects and, have a lower mammalian toxicity than other insecticides groups. Mammals generally eliminate pyrethroid rapidly by metabolic processes and excretion (178). In fact, farm workers should know more about pyrethroid and use it more. But the study shows that it is still not in much use as the knowledge about the effectiveness of pyrethroid is not realized among the study population.

In this study, the relationship between the socio demographic characteristic (age, sex, education and income level) and total pesticide knowledge level of respondents were also explored. There was no association between socio demographic characteristic (age, sex, education and income level) and total pesticide knowledge level of respondents. Most of the respondents in this study has moderate and high knowledge upon pesticides but in this study, most of the respondents (43.8%) who passed the secondary school level and they may not be aware of toxic effect of pesticide on their health which leads to inadequate and improper use of safety equipment. Another reason was, their culture and their behavior. They were very fond of performing unsafe act, which means, although they were aware of untoward effect of various pesticides, they behave themselves as skill labors, and experts which leads to false sense of safety in the other hand or another hand, such behaviors against the measures and proper practice stated in safe handling and safe production of pesticide, were dangerous on the long run, hence it should be well organized to make them become aware of the danger.

Practice level of this study, total practice score ranges from 0 to 34 and the average practice score of them was 13.23±3.11 with the range of 5 to 25. The majority of them had poor level of practice (79.2%). According to practice when mixing the pesticide, follow the bottle instruction level, majority of respondents (29.5%) did not follow instruction on package of pesticide, (13.2%) only wore using rubber gloves and string sticks and wearing PPE (48.2%) while mixing pesticides. Pesticides label are tagged in Myanmar language and the agricultural workers can understood the indication of personal protective equipment and instructions but in this study, even they could read instruction and label of pesticide packages but they still have to face from dangers about the pesticide exposure. The weakest point in knowledge and practice level of agricultural workers was proper utilization of pesticide. Farm workers in this study do not want to use PPE although they have knowledge on it because of tropical weather and inconvenient in working for long duration and some PPE are expensive and cannot buy easily. By this reason, only (12.2%) of the participant spill some pesticide on their clothes and body in early morning, changing clothes and clean body immediately for their personal hygiene habits and only (14.2%) changed the new clothes immediately after used pesticides. Utilization of personal protective equipment among agricultural workers was meet up to chemical safety level. Therefore, the agricultural workers should be trained for safe use of pesticide.

Regarding the life cycle approach of hazardous chemicals, in this study the weakest point is found relating to practice about safe disposal of pesticides. There are some (7.2%) people who know safe disposal of pesticide. (95%) of the respondents are aware of the instructions included in label of pesticide containers, they can be said to have sufficient knowledge related with pesticide label. Matthews's studied said that pesticides should be stored in their original, tightly closed containers. A pesticide storage area should be a separate building, away from people, living areas, food, animal feed, and animals. The area must be well ventilated, well lighted, dry, and secure, with lockable doors and windows (179). The majority of respondents didn't think that there was the risk of exposure during carrying and storage. Only (35%) wearing with long sleeved shirt and long pants when applying the pesticide. If they are not aware about the risk of exposure during carrying and storage, they would not careful about it and can cause danger to the environment. Therefore, training about pesticides' utilization, its storage, its toxicity and its misusages should be given to farm workers.

Regarding practice about safety measures of pesticide utilization, most of the respondents know these measures such as avoidance of eating, smoking and drinking while spraying pesticide and that pesticide should not be sprayed during the windy condition, under extreme heat of the sun and while raining. 44.2% never done lunch in paddy field and only (19.2%) obeyed never smoking, eating and drink (water) during spraying pesticide. Up wind site is the most common answer as the site, which should be started in spraying pesticide. So, there is an adequate knowledge about correct or appropriate site, which should be started in spraying pesticide (180).

When exploring practice about personal protective equipment, most of them over three quarter knew about wearing hat, cap and protective cloths but only (35%) said about gloves and rubber boot. Moreover, no one knew that apron should be wear as PPE. If they didn't concern about wearing these gloves or rubber boots, they could easily suffer pesticide toxicity when they work in farming. The reasons why farm workers are not keen on the use of protective clothing and apron are that these accessories are more expensive than hat, mask and gloves. Moreover, protective clothing and apron are not easily available. And most farm workers do not seem to realize the importance of using these things, PPE, as they think wearing masks to prevent the strong smell of pesticides and gloves to protect the skin is enough. Another reason is that the knowledge of the respondents concerning with PPE is low and not enough. Thus, they have no inclination to do correct and safe practice.

In comparison with these studies, farm workers of this study have less knowledge about PPE. The knowledge of protective clothing and aprons is not as common as masks and gloves which are more easily accessible. Besides, even in the kitchen, Myanmar housewives especially in rural area, hardly wear aprons. And the aprons intended for farmers are harder and thicker. So, farm workers are not keen on wearing aprons at work place as it is too uncomfortable to wear while working in the tropical climate. Special designs and materials should be used for making aprons as protective device. The lack of knowledge about PPE is due to the insufficient knowledge about the importance of these accessories. The more knowledge farm workers have concerning with the importance of PPE, the more protective measures they will employ and the less they will be exposed to the danger caused by the use of pesticide. No matter where and when pesticides are being used, there is a need to make sure that agriculturalists protect themselves well enough from contamination. However, weather condition such as heat and humidity may cause discomfort since most protective apparel has low heat dissipation. Moreover, farmers can not wear boots, which is an appropriate PPE, because it may damage the crops. Thus, the problem of wearing additional protective equipment in tropical countries is well recognized and has been commented upon over the years (2).

On the other hand, the respondents know about pesticide from more than one source. Of which, mass media such as TV/Video (27.25%) because of no electricity in the village and health education materials like poster/ pamphlet of education on safety measures prescribed by Myanmar agricultural service (78.5%) respectively. The majority know about pesticides from other sources of information such as from their experience, pesticide labels on containers and pesticide shops and pesticides companies. Moreover the role of health workers in providing health education is not as much as it should be. It is found that there are a few who have got the required knowledge from health personal such as doctor, health assistant and midwife. Ministry of Agriculture and Irrigation also provides some training programmes. Yet, health education by health personals is limited.

In this study, 19.2% avoid smoking, eating and drinking water while spraying pesticide. The study done by Dougall et al (1993) showed that about a quarter of 130 pesticide users smoked while using pesticide, one sixth of them ate food while handling pesticide and over 60% said that they never wore protective clothing. In comparison with the study done by Dougall et al (1993) , farm workers of this study do not take safety precautions as much as pesticides users in Saint Lucia, west Indies but the same behaviour and personal habit of pesticide users in these two studies is detected. To promote safety practice of chemical pesticides, providing knowledge to reach behaviour changes of pesticide users is one of the important factors (181). In the study done by Nwe Nwe Oo (1996), it is found that regarding the practice on safety measures more than 90% of exposed workers never smoke while handling pesticide. One-third of exposed workers used gloves and respirator while handling pesticide and 60% to 80% of workers used boots, gloves and masks while handling pesticide (129).

In this study, the relationship between the socio demographic characteristic (age, sex, education and income level) and total pesticide practice level of respondents were also explored. There was no association between socio demographic characteristic (age, sex, education and income level) and total pesticide practice level of respondents.

Farm workers who had low education level would have difficulties in understanding the warnings on the label of the pesticides bottles and while they were being educated. Therefore, special attention should be given when need based education on pesticides was delivered. Therefore, health education on pesticides should be focus on farm workers with low education, older age group and long work duration in order to promote preventive practice regarding pesticide toxicity. In this study, poor level of practice was more than good level. So health education and other essential regulatory measures should be provided to raise practice level of farm workers such as provision of training programme in collaboration with Ministry of Health and Ministry of Agriculture and Irrigation.

5.2 Effects of pesticides on health

While asking the 400 participants of ground-nut farmers(both male and female) using Pro-forma, ground-nut farmers were asking about neurotoxic symptoms, if they have to make notes about what they have to remember, whether their friends and relatives tell them that they have shorten memory, and if they have often go back and check things they had already done. The majority of the farm workers said that they felt headache at least once a week. About half of the farm workers answered that they perspired without any particular reason. And 43% of the farm workers felt abnormally tired. This result was similar Kachaiyaphum’s study(182). These neurotoxic symptoms may be associated with the pesticides’ effect on health or may be due to other factors such as heat exhaustion or life styles or socio economic factors which cannot be differentiated in this study. In the observational study, the number of family members were highest in 5 members (29%) and among the married couple, almost 2 children were (25%). A few 16 (4%) suffered and though that infertility. Therefore, this study could not differentiate the causes of these symptoms suffered by farm workers. However, this study recommended doing an experimental follow-up study to explore the effect of these symptoms.

On the other hand, there was no abnormal finding in physical examination done in laboratory study (only in 100 male ground-nut farmers). In physical examination, sign and symptoms pesticide toxicity were monitored in all system such as respiratory system, central nervous system, cardiovascular system, etc using physical examination chart and proforma. It included past history of hospitalization and reason for hospitalization. Also in this study measured Cholinesterase monitoring and it is technically feasible and necessary to protect health of the workers. Cholinesterase is essential for normal functioning of the nervous system. When cholinesterase is low because of excessive inhabitation, the nervous system may be malfunction, producing pesticide poisoning symptoms such as fatigue, light-headedness, nausea, vomiting and headache (183). Workers shown to have low level should be avoiding from further exposures to these pesticides until their cholinesterase level returned to normal.

Farm workers with low level of knowledge had more blood cholinesterase level abnormal level than the farm workers with high knowledge. These mean that low knowledge group had more exposure with pesticides and suffer from pesticides toxicity than others. They may have low concern about toxicity or preventive practice because they didn’t have much knowledge. Therefore, the farm workers who use pesticide should be given health education to promote their awareness on pesticide toxicity, using preventive measure (PPE) and monitoring blood cholinesterase level periodically.

Agricultural workers will be needed to fully inform about the compliance standard and the principles supporting it. Workers who handle pesticides will need to understand the pesticide toxicity monitoring system, how to protect themselves from them, and what they can do to minimize their exposure to pesticides. In order to do so, specialized training and workshops should be done in different layers of groups such as employer, employee, pesticide manufacturing and selling companies and shops, farm works, etc.

5.3 General Information of Participants for Laboratory Study (Phase II)

This study was to find out the effects of pesticide on male reproductive system by using biomarkers (seminal analysis, blood hormonal level and blood cholinesterase level) and also did dermal exposure assessment by skin wipe test among 100 male ground-nut who have worked with pesticides and/or to have lived in the study area more than 5 years before the study and farmers age between 18-49 years old (Reproductive Age) and sampling from observational study in Kyauk Kan village of Nyaung-U District, Mandalay Region, Myanmar between growing period at 8.6.2016 to 18.6.2016 and non-growing period at 17.12.2016 to 27.12.2016. All of the laboratory study of male ground-nut farmers were in the middle age (Mean 37.5 ± 9.45 SD) which was the same as previous studies ((154), (184), (185)). Normally, an agricultural activity in developing countries was done by men, however, there are some female involving in Mancini's study (186).Half of the respondents are primary education level with BMI (Mean 18.34 ± 2.14 SD). Body mass index (BMI) of ground-nut farmers were classified in the lower than the normal range (18.5-24.9) by WHO and most of the ground-nut farmers in this study were mild underweight (17.0-18.49 kg/m^2) as (76%), (2%) were moderate underweight (16.0- 16.9 kg/m^2) , only (18%) were within normal weight (18.5- 24.9 kg/m^2) and (4%) were pre-obese (25-29.9 kg/m^2) . All the results of BMI range according to WHO for this study were shown in Appendix H (187). Primary education level was the major group of population in this research. Safety instructions on containers are often written in unfamiliar languages, many farmers are illiterate, and the instructions themselves are difficult to follow. Most of them did not assure about routes of pesticides residues exposure and risk behaviors such as eating food without hand washing properly may pose health effects due to directly exposure via oral route. Compared to other agricultural studies of Pyae Phyo Thar (2012) (12)and Myo Min (2008) (16)the agricultural farmers in Myanmar have poor hygiene practices as well and almost all of the respondents did not use personal protective equipment (PPE) while mixing, loading and spraying with pesticides by doing questionnaire as well as observational check list. Most of the farmers did not use the amount of pesticide following the instruction because they believed that the huge amount could protect pest with high efficiency.

There were also told to all participants that all information as well as biological samples and data obtained for the study would remain confidential. Written consent to participants was voluntarily obtained from all the participants in the study prior to their participation and each participant was paid 5000 Myanmar Kyats ($ 5) to participate.

5.4 Differences in several biomarkers such as seminal analysis, serum hormone levels and blood cholinesterase levels between ground-nut farmers

5.4.1 Semen collection and seminal fluid analysis

The present study among ground-nut farmers who worked with organophosphate pesticides indicates differences in several biomarkers such as seminal analysis, serum hormone levels and blood cholinesterase levels. One of the suspects of altering reproductive function and decreased sperm count is organophosphate pesticides (OPs) (103) In this study, there were difference and statistically significant in biomarkers of seminal analysis (P value < 0.05 in $_{P}$H, Viscosity, Motility, Morphology and sperm count) between growing and non-growing period by using Wilcoxon Signed Rank test. These findings may lead to further evidence that occupational exposure to other pesticide residues adversely impact on the semen quality and the comparison of the results in semen analysis level significantly decreased in non-growing period when compared to those in growing period. The lower semen quality in pesticide applicators extensively exposed to OP pesticides is consistent with the findings in other studies related to exposure to OPs ((30) (63)). Exposure to pesticide can reduce prostate function and prostate contributes to the acid component of the seminal fluid. If prostate function is reduced, then the relative increase in the contribution of the seminal vesicles results in more alkaline seminal fluid, and an increase in the seminal $_{P}$H (188). According to this, in this study, an increase in the seminal $_{P}$H at the growing period and can be concluded that pesticide exposure can effect the prostate function and it contributes to the acid component of the seminal fluid. Some of the studies have found effects of exposures to pesticides on sperm concentration ((30) (189)). In this study; for diagnosis for sperm analysis, (74%) of male ground-nut farmers have become victim of Oligozoospermia which means that below the lower reference limit, in growing period but increased in non-growing period and resulted as (46%) because in non-growing period, the ground-nut farmers have lack of exposure to pesticide residues. Thus, we can draw a generation conclusion that sperm analysis results can be associated with exposure to pesticide residues and time duration. That means the longer the time of exposure to the pesticides, the greater the risk. During the growing period, only (1%) of Necrozoospermia can be found. That means that low percentage of live, high percentage of immotile. There was also only (1%) of Azoospermia during growing period was found. That means that no spermatozoa in the ejaculate in growing period but in non-growing period, no more Necrozoospermia and Azoospermia.

Over a past few decades, animal studies have shown that organophosphates may decrease sperm density and motility and consequently, the fertility (56). In one study about organophosphate exposure in farmers at Malaysia, Hossain et al found that men with occupational pesticide exposures had higher risks for lower semen volume, lower concentration, higher abnormal morphology and decreased sperm motility (98). One of the organophosphate study in Mexico , Recio-Vega, R., Ocampo-Gomez, G., et al identified that exposure to OPs was associated with lower the seminal volume, greater as seminal $_{P}$H and decreased sperm count for men in the highest exposure group (OP sprayers) (63). Our finding is similar with Hossain.F et.al and Yucra.S et.al finding that pesticides non exposure groups had decrease in number of subjects with abnormal pH ((98) (101). This may be happened in situations in which sex accessory-gland function is transmuted. Seminal vesicles and prostate contribute 60% and 30% of the seminal volume, respectively (190). The parameters such as seminal volume, seminal $_{P}$H, sperm motility, and sperm morphology may be affected in OP applicators independent of an effect upon the testis. Reduction of sperm motility has been related to reduce function of the seminal vesicles and the prostate; high levels of immature sperms could be due to high concentration of the muscles in the epididymis; seminal volume and seminal $_{P}$H may be affected by dysfunction of the seminal vesicles and the prostate (35, 36). In this study exposed that pesticide applicators have altered semen, probably at the level of epididymis, seminal vesicle, and the prostate function and these alternations may result in low sperm motility and high abnormal sperm morphology and sperm count concentration.

Pesticides, mainly the organophosphate compounds are known to affect the general health. In spite of a number of experimental studies, evidence for the adverse effect of pesticide exposure in human subjects is exiguous. This may be due to the major poorly educated farming folks and their cultural beliefs hindering the collection of semen samples for the cause of research. This study efforts in the direction among the ground-nut farmers in Kyauk-Kan village, Nyaung-U District, Mandalay Region, Myanmar, have yielded a response which may form a scientifically a basis for future studies and a provide a statistically a sound sample number for investigating of hypotheses about pesticide exposures of farming men. The WHO guidelines for the examination of human semen were strictly followed (149) (150) for the semen analysis and all the semen specimens were evaluated within on hour of collection and analyzed by qualified personnel. The interviews were performed privately and strict confidentiality was maintained. All related biases were ruled out, and the interviewers were not informed of the semen characteristics of the farmers before they were being interviews. There was no chronic disease, especially testicular diseases among the farmers who participated in this study. The percentages of risk behaviors such as smoking, and drinking alcohol were found in the farmers. All the semen parameters showed significant differences between growing and non-growing period. Increased in sperm abnormality and declines in both motility and sperm numbers correlate well with the experimental rodent studies (191) (192) confirming the adverse effect of pesticide exposure on the pesticide users.

All the parameters of semen analysis showed significance differences between growing and non-growing period. There is an evidence of reduction in semen quality or low sperm count associated with exposure to agricultural pesticides (193). The present results provide sufficient evidence for concern that there is a strong relation between pesticide exposure and male semen quality. In the recent years, more and more evidence indicates that pyrethroid insecticides can also reduce sperm count and motility, cause deformity of the sperm head, increase the count of abnormal sperm, damage sperm DNA and include its aneuploidy rate, as well as affect sex hormone levels and produce reproductive toxicity like organophosphate (194). Exposure to pesticides lowers sperm levels well below the limit for male fertility. Although, it is proved that pesticide exposure is associated with infertility, there are not large-scale studies assessing their relationships to human infertility. The exact role of male mediated toxicity on such adverse effects like abortions, congenital malformations, pre-term delivery etc. is not yet fully understood. The exciting data on various endocrine disrupters and risk factors (malnutrition and infections which can exaggerates the risk) suggest a greater vulnerability of the population of developing or underdeveloped countries to reproductive hazards from exposure to these pesticides. Thus, there is a need to constantly monitor both exposure and affect parameters such as congenital malformations, subnormal growth and development, testicular cancer and trend of semen quality in a given population and ethnic groups. Further, the general public needs to be educated for vigilant use of the pesticides. The risk assessment to the human is absolutely necessary for the pesticides that have already proven to be toxic to the reproductive system in animal studies (57),(58).

5.4.2 Blood Collection and Reproductive Hormone Assay

In this study, there were a difference and statistically significant in serum hormone level (P value < 0.05 in Follicle-stimulating hormone and Testosterone) in growing period and non-growing period Wilcoxon Signed Rank test respectively. In normal mechanism, Testosterone, produced by the Leydig cells located in the interstitium of the testis, is essential for growth and division of the testicular germinal cells, which is the first stage in forming sperm (37). Luteinizing hormone, secreted by the anterior pituitary gland, stimulates the Leydig cells to secrete testosterone (36).

The changes in sex hormone levels that were found in pesticide applicators have to be considered with regards to physiological feedback. Sexual and reproductive function in man are the result of neuroendocrine mechanisms involving hypothalamic, pituitary and gonadal hormones. The male reproductive system is dominated by the sex steroid hormone testosterone produced by the gonads in testicular Leydig cells. The rate of secretion of testosterone is enhanced by LH secreted by the anterior pituitary gland. The LH secretion rate in turn is enhanced by gonadotropin releasing hormone (GnRH) which is released in the hypothalamus and is under negative feedback control by testosterone. Receptors for LH are present on Leydig cells. The role of LH is to induce the biosynthesis and secretion of testosterone. In addition to gonadotropins and gonadal steroids, other peptide hormones, prolactin also plays a role in the reproductive system. Testosterone after being released from Leydig cells diffuses through the interstitial lymphoid spaces and reaches the seminiferous tubules. Testosterone is one of the key regulators of spermatogenesis. It is assumed that high amounts of testosterone are needed to initiate spermatogenesis. FSH is the second key regulator of the spermatogenic process. Its receptors are present on the Sertoli cells. Neither testosterone alone nor FSH alone are capable of bringing about a quantitatively normal spermatogenesis. Both testosterone and FSH exert an initial stimulatory effect on the same germ cell type (renewing stem cells) (36, 37).It is assumed that they interact at the receptor level by each of them stimulating the synthesis of the receptor for the other hormone. The aromatase system stimulated by FSH catalyzes the formation of estradiol from testosterone (38). Whenever secretion of testosterone becomes too great, this automatic negative feedback comes into effect, operating through the hypothalamus and anterior pituitary gland, reduces the testosterone secretion back toward the desired operating level (21). When the seminiferous tubules fail to produce sperm, secretion of FSH by the anterior pituitary gland increases markedly. Conversely, when spermatogenesis proceeds too rapidly, pituitary secretion of FSH diminishes (37).

Toxic exposures can alter the production, release, or function of hormones that regulate spermatogenesis. Decreases in testosterone can also affect a man's desire for, and ability to perform, sexual intercourse. Some chemical or physical agents can damage a man's testicles, the sperm cells, or the mature sperm. This damage can cause a reduction in the number of sperm produced, a total absence of sperm, changes in their shape or ability to move. Exposures of men to workplace chemicals can also affect reproduction and development if the agent is secreted in the seminal fluid (195). Utilization of pesticides without safety precaution results in adverse effects on health. It is necessary to have insight to the knowledge and practice of farm workers in using pesticides.

This study has some results that carried conflict of interest with results of prior studies. Regarding hormones, it was observed that serum testosterone and FSH were significantly reduced in the growing period, whereas LH were not different but increased abnormal rate in growing period too. However, neither testosterone nor FSH and LH levels were significantly associated with Op pesticides effect.

Similar results are found in the literature: serum LH concentrations of 54 exposed workers in a lindane-producing factory were significantly higher than in controls, also FSH without significance, and testosterone concentrations in serum were found to be slightly lower (196). The comparison of occupational categories revealed a significant difference in the prevalence of agricultural occupation (reported with long-term exposure to pesticides) in a fertility study (197).

Rogelio Recio et al pointed out their study of Mexican agricultural workers discussing that low serum LH and FSH levels were significantly associated with OP urinary metabolites (169). However, neither testosterone nor estradiol levels were associated with metabolites. According to both studies, it looks as if pesticides affect hypothalamic-pituitary endocrine function. As serum LH was affected, the low concentration of serum LH may have resulted in a low stimulation of the Leydig cells and a low production of testosterone in this research. However, another researchers, Padungtod C et al presented their findings that serum LH was increased in male workers who exposed to organophosphate pesticides whereas serum testosterone was reduced (30). This latter study suggests a direct effect of on the testis, since low testosterone production may increase serum LH as a negative impact. All the effects observed in the present study could have been regarded as the consequence of this exposure, although other unmeasured lifestyle factors could also play roles. The use of protective equipment (i.e, thick protective pants) has been suggested to be related to higher scrotum temperature and heat stress of the testes (198). In this study, 100% of the pesticide applicators did not use any protective measure by observation check list and making heat stress a less probable explanation for this study. The major route of exposure in agricultural is dermal and the failure to use protective equipment is probably a main cause of OP exposures.

Another Study, Quinalphos, another commonly used OP insecticide reduced prostatic acid phosphatase activity and the content of fructose in the accessory sex glands as well as plasma levels of testosterone, FSH, and LH. Quinalphos is therefore supposed to have suppressive effects on the function of the accessory sex glands by inhibition of the release of pituitary gonadotropins (61).

One of the animal study in adult male rats, Ferdinand Ngoula et al. discovered that cholesterol is the main precursor for steroidogenesis and it is produced mostly in the liver from LDL and HDL(199). The decrease observed in the serum was correlated with the effect of organophosphate pesticides exposure on the liver as shown by the significant reduction of body weight. On the contrary, the increase of cholesterol level in the testes leaded to reduce the production of testosterone, the main hormonal involved in the control of fertility of animals including rats (200).

This study gave contradictory results, and interpretation was rendered difficult because hormonal values vary according to the time between the last toxicant exposure and the time of hormonal evaluation(201). The hormonal disruption in agricultural workers in the present study, together with results from other experimental animal studies, suggests that OP exposure disrupts the hypothalamic-pituitary endocrine function and also indicates that FSH and LH are the hormones affected. This study conclude that even a very low level of pesticide exposure may affect fertility by the results of seminal analysis, (74%) got the results of Oligozoospermia which means that below the lower reference limit. Finally, the conclusions should promote further evaluation of male reproductive toxicity of commonly used substances or those that are likely to be in contact with human populations, on male fertility.

5.4.3 Blood Cholinesterase testing

Blood cholinesterase was used as biomarker of pesticide exposure. Specimens was collected from male ground-nut farmers by finger-prick technique (require only 10 µl of blood) after the end of their shift. It was analyzed by Test-mate ChE Kit (Model 400) that was manufactured by EQM Research, Inc because most organophosphate and carbamate chemical class can affect the blood enzymes acetylcholinesterase (AChE), and plasma cholinesterase (PChE).

In this study, there were a difference and statistically significant in blood cholinesterase level (P value < 0.05 in Hemoglobin adjusted acetyl cholinesterase: (HAChE) and Plasma cholinesterase (PChE)) in growing period and non-growing period by Wilcoxon Signed Rank test. In addition, the study assumed dermal exposure with pesticide residues in growing period was higher in ground-nut farmers and more affected with acute action. However, the findings indicated that ground-nut farmers were exposed to pesticide residues showed that the results tended to chronic exposure with pesticide residues and it might to be chronic health effect problem.

The blood cholinesterase levels among ground-nut farmers were found to be highest in growing period. It revealed that the average acetyl cholinesterase (AChE) level, haemoglobin adjusted AChE (HAChE), and the plasma cholinesterase (PChE) of them were 3.33±0.75U/ml, 12.94 ± 1.76 U/g Hgb, and 1.64± 0.47 U/ml, respectively. The levels of the current study were higher than the other study of Pidgunpai et al found that the average AChE level and PChE level of farmers was 2.9±0.6 U/ml and 1.6 ± 0.3 U/ml, respectively (30). According to the results of study at Padung Krung Kasem market, Bangkok in 2015 by P. Ong-artborirak , AChE (HAChE) was 3.00±0.57 U/ml, and plasma cholinesterase (PChE) was 1.98±0.49 U/ml and both results were very similar to the findings of frequency in this study (156). According to another study of Wilaiwan and Siriwong, it was investigated that cholinesterase level in blood among 35 rice farmers who were exposed to organophosphate pesticides was at 2.63 U/ml and 35 non-farmers in 2014 at Sisa Krabue sub-district, Ongkharak district, Nakhon Nayok province were at mean of 2.80 U/ml (168). Neupane et al. examined that acetyl cholinesterase levels (AChE) among 90 vegetable farmers and 90 controls in Nepal by using Test-made ChE (Model 400) and AChE were significantly lower among farmers (mean = 3.35U/ml) as compared to the controls (mean = 3.64U/ml) (202).

Body mass index (BMI) of ground-nut farmers was 18.34 and it was classified in the lower than the normal range (18.5-24.9) by WHO (187). There are suggestions that dietary factors can influence cholinesterase levels. Low levels of cholinesterases have observed in malnutrition (203). However, the findings revealed that ground-nut farmers were exposed to pesticide residues. Sometimes, there was a minor error of temperature range during testing blood period and it might be influenced the performance of the Test-mate cholinesterase kit but average temperature at the tested room in both periods of growing and non-growing was almost the same. Therefore, farm workers with longer than ten years duration of work had more below normal blood cholinesterase level than farm workers with ten years duration. it show that farm workers with more than ten years of work durations needed to monitor their blood cholinesterase level regularly before and after handling or using pesticides. Moreover, they should be avoided handling pesticide if their cholinesterase level is not reach to normal within 14 days.

Ratner et al revealed that decrease in blood cholinesterase activity among residents during the summer, and this seasonal variation was explained by the ingestion of pesticide residues left in fruit and vegetables (204). In that event, cholinesterase level monitoring are useful to prevent or reduce exposure to pesticides. The key to reducing health hazards when using pesticides was to always limit the farmer's exposure by wearing PPE and used a low-toxicity pesticide when available. Reading the label and practicing safe work habits were minimized hazards from the use of pesticides (111).

The farmers who use organophosphate pesticides in the present study were neurotoxic in nature, thus the AChE activity was likely found to be depleted and the PChE activity were found to be slightly depleted and it is widely accepted that AChE and PChE are biomarkers for organophosphate pesticide exposure which can be understood that the obstruction activities of AChE and PChE are due to organophosphate pesticide exposure among the ground-nut farmers. Farmers are directly exposed to pesticides in a variety of ways.

Environmental exposure to OP pesticides along with unfavorable reproductive results in both men and women working on the farms are more and more reported all over the world. No matter if it is exposure to OP below the level that leads to clinical manifestations of severe OP toxicity, this leads to a detrimental effect on fertility, growth as well as development. In this connection, the evidence regarding weakened fertility exists because of a decrease in semen quality as well as possible less testosterone levels in exposed men (205). In this study, semen quality as well as testosterone levels in exposed OP pesticides duration of growing period in each results were low and it can conclude that there is a connection of OP pesticides exposure and it can alter the reproductive function by reducing brain acetylcholinesterase activity and monoamine levels, thus impairing hypothalamic and/or pituitary endocrine functions and the gonadal processes (169).

Thus, occupational exposure to pesticide residues may pose acute and chronic effect via dermal and oral routes among ground-nut farmers, while good personal hygiene practices may prevent and reduce the health effects from pesticide residue exposure. Pesticides products can be splashed or spilled on exposed skin during pouring, mixing process, and during application, when spray or dust can contaminate the exposed skin or clothing. There is a risk of inhalation hazard since most pesticides are not sufficiently volatile, or due to the particles sizes generated during conventional application of sprays or dusts.

5.5 Health symptoms from exposure to pesticide residues

In Myanmar, organophosphate groups, organochlorine groups and carbamate groups are used commonly. But in this study, most of the ground-nut famers used organophosphate group and pyrethroids group. Pesticides are used in combination of at least 2 to 3 and sometimes up to 5 kinds are mixed and sprayed on the crops. These pesticides are used deliberately without any restrictions and precautions and also the empty containers are not disposed-off systemically due to scarcity of the land areas (16). The ubiquitous organophosphates present a continuing health hazard in agricultural, public health eradication programmes and as chemical warfare agents. Most of the ill-health following exposure to organo-phosphorus compounds has been attributed to the inhibition of cholinesterases (206). Organophosphate pesticides (OP) are suspected of altering reproductive function by reducing brain acetylcholinesterase activity and monoamine levels, thus impairing hypothalamic and/or pituitary endocrine functions and gonadal processes (169). Organophosphate pesticides has the acute cholinergic action and sign and symptoms were; at muscarinic sites which causes an increase in secretions (bronchorrhoea, salivation, tearing and sweating), bronchoconstriction (tightness in the chest and wheezing), bradycardia, vomiting and increase in gastrointestinal motility (abnormal tightness, cramps and diarrhea). Organophosphates cause the diagnostic miosis in the eye, which results in blurred vision. Nicotinic sites (e.g. neuromuscular junctions), which cause muscle fasciculation and a flaccid paralysis in severe exposures. Within the central nervous system, which causes headache, insomnia, giddiness, confusion, drowsiness and, in severe exposures, slurred speech, convulsions, coma and respiratory depression (207).

In this study detected some of the symptoms related to nicotinic action of organophosphates on central nervous system and on neuromuscular activity. These symptoms are dizziness, headache, weakness , blurred vision and are of high prevalence, whereas the muscarinic effect such as sweating, hyper salivation and tremors are found and highest in growing period than non-growing period. The most frequent symptoms in growing period were blurred vision (27%), followed by dizziness (13%), headache (12%) and sweating (6%) but all are slightly decrease in non-growing period.

Diagnosis of the cholinergic syndrome in most instances is based on clinical features. Miosis in combination with fasciculation is pathognomonic of this syndrome, particularly in adults. Lachrymation, salivation, bronchorrhoea and excessive sweating along with bradycardia provide supportive evidence. Sulphurated organophosphorus agents possess a pungent garlic-like odour that is easily recognized by clinicians. This odour when present in the breath, vomitus or clothing is often the main diagnostic tool in developing countries where in the majority of instances the agent implicated in poisoning is not known with certainty.(208). However, because of the limitation, cholinesterase estimations remain the only useful biomedical tool in organophosphate exposure at present. However, the interpretations of these estimation are not without problems. There are many causes of decreased activity of cholinesterase that are not related to exposure to organophosphates and carbamates (anticholinesterases). Theses are genetic, physiological (age, gender,pregnancy, etc.), iatrogenic (therapeutic agents), disease states, exposure to smoke fumes and in some instances of uncertain origin (209).

Neupane et al. mentioned that most of the vegetable farmers suffered as blurred vision (50%), extreme tiredness (47%), excessive sweating (43%), headache (40%) and muscle cramps (40%), respectively (202). Similarly with Ngowi et al. revealed that excessive sweat, headache and dermal effect were more commonly reported (210). Wilaiwan's study (168) and Kachaiyaphum study (182) revealed that common pesticides related symptoms that farmers suffered were headache and dizziness. The study of P.Ong-artborirak found that blurred vision (29.7%) , dizziness (26.4%) and muscle twitching or cramps (19.8%), excessive sweating (18.7%) and which as same as the most frequency reported with this study (156).

Lu, J conducted a study on illness related to pesticide exposure among cutflower farmers in La Trinidad, Benguet. The study used personal physical health, laboratory examinations and questionnaire on work practices and illness as a measurement. Majority of exposed farmers, male gender, were symptomatic, with most common complaints being headache (48%), easy fatigability (46.1%) and cough (40.2%). An analysis showed that RBC cholinesterase levels were positively associated with age, selling pesticide containers, number of years of using pesticides, use of contaminated cloth, incorrect mixing of pesticides and illness due to pesticides. Significant associations were also found for haemoglobin, haematocrit, RBC, white blood cell and platelet count (211).

Mitoko et al investigated on assessing health hazards related to handling, storage, and use of pesticides, on agricultural estates and small farms. The 256 exposed subjects and 152 controls were completed questionnaire on symptoms experienced at the time of interview including sex, age, main occupation, and level of education. Symptoms on health effects of pesticides that inhibit cholinesterase (organophosphate and carbamate) reported during the high exposure period. Symptom prevalence in exposed subjects was higher during the high exposure period than the low exposure period, however no statistical significant was found. A clear and significant change in symptoms prevalence was found in controls with a higher prevalence in the low exposure period. The relation between cholinesterase inhibition and symptoms showed that prevalence ratios were significantly >1 for respiratory, eye, and central nervous systems for workers with >30% inhibition.(212)

Strong et al studied on 21 farmworkers in eastern Washington to assess the relationship between self-reported health symptoms and indicators of exposure to OP pesticides. The diagnosis of health symptoms most commonly reported included headache (50%), burning eyes (39%), pain in muscles, joints, or bones (35%), a rash or itchy skin (25%), and blurred vision (23%). The proportion of detectable samples of various pesticide residues in house and vehicle dust was weakly associated with reporting certain health symptoms, particularly burning eyes and shortness of breath. However, no significant associations were found between reporting health symptoms and the proportion of detectable urinary pesticide metabolites (213).

The neurotoxic symptoms may be associated with the pesticides' effect on health or may be due to other factors such as heat exhaustion or life styles or socio economic factors which cannot be differentiated in this study. Taneepanichskul , N studied that most chili farmers mentioned their health effects related to Central Nervous System (CNS) (67). This study could not differentiate the causes of these symptoms suffered by farm workers. However, this study recommended doing an experimental follow-up study to explore the effect of these symptoms. On the other hand, there was no abnormal finding in physical examination. In physical examination, sign and symptoms pesticide toxicity were monitored in all system such as respiratory system, central nervous system, cardiovascular system, etc using physical examination chart and proforma. It included past history of hospitalization and reason for hospitalization.

Reporting health symptoms in growing and non-growing period, it was found that there were significant difference in dizziness and blurred vision under central nervous system (p-value <0.05) in this study. The results were similar to previous studied showed that the predominance of eye symptoms found in expose group was significantly higher (p< 0.01) in Wilaiwan study(168). During agricultural operations, farm workers' eyes could be exposed to pesticides while spraying if there is a lacked of proper preventive steps. As a result, these chemicals are being absorbed through the eye tissue and enter the blood circulation. Exposure of unprotected eyes to pesticides results in the absorption in ocular tissue and potential ocular toxicity (214).

The present study found that the positive associations of farmers are significantly related to central nervous system symptoms. It is reasonable to show a health report regarding the symptoms caused by organophosphate poison to insects and mammals mainly by phosphorylation of the acetylcholinesterase enzyme (AChE) at nerve endings. The consequence is a loss of existing AChE which makes organs become over motivated by the incremental acetylcholine (Ach, the impulse-conveying substance) at the nerve ending. The enzyme is vital to regular control of the transmission of impulse from nerve fibers to smooth and skeletal muscle cells, glandular cells, as well as autonomic ganglia and within the central nervous system (CNS) (215).

5.6 Factor associated between biomarkers (Blood Cholinesterase Monitoring, Blood Hormonal Assay and Sperm Count) and work related factors in Growing Period and Non-Growing Period

Binary logistic regressions analysis adjusting between biomarkers (Blood Cholinesterase Monitoring , Blood Hormonal Assay and Sperm Count) in growing and non-growing period with work related factors such as age, education, working hours, working years, smoking at work, eating and drinking at work , alcohol drinking at work , use of PPE and washing hands after working. Firstly, AChE ,was an association between smoking at work and eating/ drinking at work are statistically associated with AChE in growing period (p-value < 0.05) but there was no statistically significant association between working years with AChE in non-growing period. For HAChE level, there was not statistically significant association in both growing and non-growing period. The study of P.Ong-artborirak found that washing hands during their work could increase the haemoglobin adjusted acetyl cholinesterase level of greengrocers about 2.55 U/g Hgb (p-value <0.05) (156).

In PChE level, there was no statistically significantly association in growing period but there was a statistically association between age with PChE level in non-growing period (p-value <0.05). Compare to the studies of Ntow et al in 2009 (216) and Wilaiwan et al. in 2014 studies (168), both AChE and PChE levels were not significant with age and education but in this study, there was an association between age in non-growing period in PChE level.. The study in 2008, Catano et al. made multivariate analysis for finding the relation between PChE activity and working years of pesticides exposure and concluded that chronic exposure to chemical compounds might lead to a higher enzyme activity and reduce OP binding to biological targets (177). In this study, both AChE, HAChE and PChE levels were not associated with agricultural works, farming characteristics of pesticide use: use of PPE and personal hygiene: hand washing. The possible reasons can conclude that the farmers were good knowledge upon how to use the pesticide and can read the label but they do not always follow the right ways and even they reported that they use PPE and as well as hand washing after the working but may be incorrect ways of their personal hygiene and the pesticides still exposed in their skin. So this study was not associated with pesticide use and wrong way of personal hygiene among the farmers and still increasing the risk and level of exposure because of improper use of PPE will make it useless.

FAO devised the guidelines for short messages of how to use personal protection of pesticide for operators against exposure in all forms that has been laid on avoiding skin contamination as well as from inhalation. The basic principle for reading the label before use and do practically on proper use for label recommendation and it can reduce or minimize direct exposure of the skin, nose, mouth or eyes when handling pesticide products, hence this reduces the personal contamination. So personal protective equipment are required in pouring, mixing and loading pesticide formulations eg, gloves and eye protection. For avoiding from inhalation of vapor, fine dust or spray, protective measures such as face masks, aprons, and hats should wear. The crucial way of personal protection is good hygiene. When working with pesticides, the farmers are not recommended to eat, drink or smoke during in working time and suggested way for washing their hands after working (2).

Factor association between blood hormonal assay with work related factors such as age, education, working hours, working years, smoking at the work, eating/drinking at the work, alcohol drinking at the work, use of PPE and washing hands after working were checked in both growing and non-growing period. There were statistically association between 1) working hours with FSH abnormal level in growing period but there was no statistically significant association in non-growing period of FSH level, 2) age with LH level in growing period and eating/drinking at the work were associate with LH level in non-growing period, 3) smoking at the work with Testosterone level in growing period and education with Testosterone level in non-growing period. Compared with other research of John D. Meeker et al studied have reported that pyrethroid insecticides affect male endocrine and reproductive function among 161 men from an infertility clinic between 2000-2003 and measured serum reproductive and thyroid hormone levels, were positively associated with FSH (all p values for trend < 0.005). Statistically significant or suggestive positive relationships with LH were also found. And also associated with testosterone and free androgen index (the ratio of testosterone to sex hormone binding globulin; p for trend = 0.09 and 0.05 respectively) and suggested for further research was need for a better understanding of the potential association between pyrethroid insecticides and male reproduction (136). But in this study, most of the participants used mixed together organophosphate (chlorpyrifos) and pyrethroid (cypermetrin) together, so that we could not differentiated which pesticides more effect for blood hormonal effect in each of the participants but we assessed direct exposure of skin contact by dermal wipe test for chlorpyrifos exposure and we can point out that there is a risk in pesticide exposure in this community. Feroz Hossain et al studied among male farmers from 3 different communities in Sabah, Malaysia at 2013 for determining the relationship between semen quality and exposure to pesticide residues. The resulted of mean values of volume, $_{p}$H, sperm concentration, motility and WBC count were significantly less in the exposed group than in compared with the non-exposed group, with p values of less than 0.05. The comparison between semen qualities such as low sperm count, motility and higher percentage of sperm abnormality of those exposed to different types of pesticides (paraquat and malathion) showed no significant differences (137).

This study found that there was no statistically significant association of work related factors with Sperm count in both growing and non-growing period. According to the association result, this study can conclude that habit of bad personal hygiene can expose to the pesticides from different pathways and the more risk effect can get the health effect form chemical exposure to human. Feroz Hossain et al studied that no significance differences were seen in semen quality between smokers and non-smoker group between those taking alcohol and those who did not(137). Cummings DE study revealed that spermatogenesis occurs in the seminiferous tubules during active sexual life as the result of stimulation by anterior pituitary gonadotropic hormones, beginning at an average age of 13 years and continuing throughout most of the remainder of life but decreasing markedly in old age (217). Two separate gonadotropic hormones, follicle-stimulating hormone and luteinizing hormone, control growth of testes, as well as their hormonal and reproductive activities. The precise mechanisms that control secretion of growth hormone are not fully understood, but several factors related to a person's secretion: (1) starvation, especially with severe protein deficiency; (2) hypoglycemia or low concentration of fatty acids in the blood; (3) exercise; (4) excitement; and (5) trauma. For instance, the extremely high levels of growth hormone that occur during starvation are closely related to the amount of protein depletion. Under severe conditions of protein malnutrition, adequate calories alone are not sufficient to correct the excess production of growth hormone. The protein deficiency must also be corrected before the growth hormone concentration will return to normal (218). In this study, the body weight (BMI) of the male ground-nut farmers were 18.34 and it was classified in the lower than the normal range (18.5-24.9) by WHO (187). So this study can predict that improper nutrition status can be associated with growth hormone depletion as well as protein deficiency and can reduce the growth hormone.

Melissa J. Perry reviewed among 20 studies on effects of environmental and occupational pesticide exposure on human sperm. Among 20 studies ; 13 studies reported an association between exposure and semen quality; 6 studies evaluating DNA damage, of which 3 reported an association with exposure; and 6 studies assessing sperm aneuploidy or diploid, of which 4 reported an association with exposure (138).

In two times testing, all of the ground-nut farmers' results were not too difference and still contaminated with pesticides. Therefore, the ground-nut farmer used of pesticides should be controlled by the government. A system for monitoring pesticide residues was needed to be established. Moreover, education, training and information activities on pesticide safety should be established and strengthened.

5.7 Health Risk Assessment

Dermal exposure was a primary route of exposure in pesticides; so farmers in paddy fields should focus on the pesticide residues, which deposit on the skin and absorb into their bodies. The three categories to estimate dermal contact are: surrogate skin techniques, chemical removal techniques, and fluorescent tracer techniques (155). This study aims to determine the dermal exposure to chlorpyrifos among male ground-nut farmers and assess the health risk of those farmers to chlorpyrifos through chemical removal techniques.

The reasonable maximum exposure (RME) at the upper confidence (95^{th} percentile) level of the arithmetic average concentration was estimated pathway to protect farmers from the high dermal exposure level in this area because the uncertainly associated with any estimation of exposure concentration may occur (153). An estimated of average concentration is used because; (1) carcinogenic and chronic non-carcinogenic toxicity criteria are based on lifetime average exposures and (2) average concentration is most representative of the concentration that would be contacted at a site, over time (174)

The average daily dose (ADD) was used to evaluate the exposure for noncarcinogenic effects. Due to unpredictable associated with any assessment of exposure concentration, the upper confidence limit (95^{th} percentile) on the arithmetic average mean was operated to evaluate those individuals who may be at the higher end of the exposure distribution (174). Contact rate reflects the amount of perverted medium contacted per unit time or event. It may vary with time of year and crop season (139). Based on the statistical calculation remarking farmers interviews (n=30). The 95^{th}

percentile of exposure duration was 19.2 years, exposure frequency was 100.27 days/years, and the body weight was 57.72 kg (at mean). Exposure assessment showed the average daily dose ADD (mean) of the ground-nut farmers at 3.66×10^{-5} mg/kg-day and ADD (RME) was 9.93×10^{-5} mg/kg-day in the growing period among 30 randomly selected samples of the ground-nut farmers. ADD value was huge in this study because duration of exposure was higher. For non-carcinogenic risk characterization, a Hazard Quotient (HQ) was used to estimate the risk and calculated by this following equation, which was provided from (166).

The last step of the human health risk assessment is the risk characterization. It includes qualitative and quantitative data, which is a tool to link with the risk manager or decision makers. In this study, HQ at mean and 95^{th} percentile RME level were 0.12 and 0.33 respectively. Based on this study, hazard quotient pointed out less than the recommended amount and there is no concern for potential non-carcinogenic effect from dermal exposure to pesticide among ground-nut farmers and no risk. Moreover, the current study investigated only 30 randomized samples for hand-wipe who sprayed Chlorpyrifos from 100 subjects of laboratory study, so that a large enough sample of applicators was not available for analysis resulting in statistical power restriction. As a consequence, the findings from this study might not be generalized to other communities and this study only looks at hands, which is a very small percentage of dermal exposure.

Taneepanichskul et al. found that ADD of dermal exposure to chlorpyrifos in chili-growing farners in Hua-rua sub-district, Ubon Ratchathani province was 2.51×10^{-9} mg/kg/day that was lower than this study and also revealed that chili farmers in Ubon Ratchathani province had an HQ < 1 associated with dermal exposure to pesticides such as chlorpyrifos and suggests that pesticide application during different farm activities may affect the level of exposure among farmers(219) (219) . And Lappharat S et al (2014) that ADD of rice-growing farmers through skin exposure of male and female was 2.59×10^{-6} mg/kg/day and that was also lower than this study and HQ of rice-growing farmers were not higher than 1.0 (HQ= 1.73×10^{-3}) and same with this study. Also similar with the study of Curwin et al., conducted on farmers in Iowa, United State and found most of hand wipe samples were no encounter (220). Compared with Jaipieam (2008) revealed that ADD of skin exposure to chlorpyrifos in vegetable growers in Thailand was 3.23×10^{-5} mg/kg/day, which was greater than this study (157).

P.Ong-artborirak et al indicted that Chlorpyrifos which is an organophosphate pesticide were detected and showed lower dose and health risk (ADD = 1.13×10^{-7} mg/kg-day, HQ = 3.77×10^{-4}) and this study done only upon dermal exposure to pesticide residues on vegetables among greengrocers in fresh market so that it may reduce the exposure of pesticide than in growing site studies(156). Richardson & Miller mentioned that Chlorpyrifos is ten times more toxic via oral administration (210). Jaipieam's study revealed that rice crops do not depend upon seasonal effect too much (157) but in this study were studied in wet season, higher HQs would be expected as hotter and wetter climate may increase pest growth. Qualitative analysis exposed that risk was found to be highly variable with exposure all in terms of farmers' years of exposure, weeks and hours of work. Other factors like as smoking status, education level and personal hygiene of individuals might be contributing factors as well. In this study, HQ chlorpyrifos of participants values were less than the acceptable level 1.0, therefore, the study suggested that the ground-nut farmers might not get a higher risk from ground-nut consuming that contaminated with chlorpyrifos.

For risk communication, it was clearly mentioned that "an interactive process of exchange of information and opinion among individuals, groups and institutions. It involves multiple messages about the existence, nature, form, severity or acceptability of health risk". Risk communication plan must be sound, with effective strategies, monitoring and evaluation to ensure the desired objectives are achieved (33). In this study, the development of risk communication materials was hand books with communicated using PPE picture for encouraging ground-nut farmers to realize and concern their health in Appendix G.

Use of pesticides is widespread in several different industries and exposure presents a significant health risk to workers involved in the end use of pesticides. The majority of pesticide absorbed into the body comes from dermal exposure, and PPE in the form of appropriate gloves and clothes has been shown to reduce absorption. However, compliance among the majority of occupationally exposed pesticide end users appears to be poor. The reasons for poor compliant are not clear and, although training appears promising, there is poor understanding of the delivery modes, content, and teaching methods that are most effective.

In term of generalizability, this study could be generalized to other agricultural area due to difference method of applying pesticides, good manner of handling and practicing of pesticide and PPE usage, such as not storing pesticide at home and washing hands after the works, could be introduced and suggested to other area by risk communication material; books and/or manuscripts. For policy implementation, this study could be suggested that the government should concentrate on dermal exposure.

CHAPTER VI
CONCLUSIONS

6.1 Conclusion

Myanmar is an agro-industrial country, the majority of country's economic sector is agriculture. Majority of country's work force consists of agricultural workers. Pesticides poisoning is one of the agricultural hazards in our country. Utilization of pesticides without safety precaution results in adverse effects on health. It is necessary to have insight to the knowledge and practice of farm workers in using pesticides. Health hazards of pesticides can be reduced by proper understanding of safety measures of pesticide utilization and practice of using personal protective equipment. The main objective of this study is to explore knowledge and practice on safe use of pesticide among farmers and to find out the effects of pesticide on male reproductive system by using biomarkers among male farmers in Kyauk Kan village of Nyaung-U District, Mandalay Region, Myanmar. It consists 2 cross-sectional studies: observational study (Phase I) and laboratory study (Phase II and III).

1. For observational study (Phase I) included 400 participants with both male and female from 182 households by simple random sampling between total number of 818 people (Male = 366, Female = 452) and about two-third of the respondents was male so the number of males was greater than females. In Myanmar, most of the farm works were usually done by male workers. Nearly half of the respondents had 10-12 years work duration. Average duration of the work was 10 years which mean that these farm workers had used with pesticide for ten years. The number of years of using pesticide was higher because ground-nut growing is a major work in the area. Finishing primary school, they started working in ground-nut industry right way. Most of the farmers applied pesticides to their fields about 5 hours per day in the paddy fields and this information indicated that the farmers who work in paddy field could be exposed to pesticides. Most of the farmers who mixed, loaded, and sprayed pesticides by themselves was 51.2%.

2. For laboratory study included 100 male ground-nut farmers from 18 to 49 years of reproductive year who are exposed with pesticides in their farms by simple random sampling from the total number of 366 males of observational study for phase II. All of the laboratory study of male ground-nut farmers were in the middle age (Mean 37.5 ± 9.45 SD). Half of the respondents are primary education level with BMI (Mean 18.34 ± 2.14 SD). Body mass index (BMI) of ground-nut farmers were classified in the lower than the normal range (18.5-24.9) by WHO (187). Primary education level was the major group of population in this research.

3. All participants were ground-nut growing farmers who always used chemical pesticide that depended on symptoms of pest. Most of the farm workers know two to three types of chemical pesticide for their plants. They spray chlorpyrifos four or five times for one crop by using sprayer connected with motor tank might. Moreover, they always used large amount of pesticide (exceed pesticide label) and mixed a different kind of pesticides together. All of them never have tested pesticide residues contaminated on their body before. In addition, they did not wear personal protective equipment such rubber gloves or boots to protect them from chemical pesticide. The respondents know about pesticide from more than one source. Of which, mass media such as TV/Video (27.25%) because of no electricity in the village and health education materials like poster/ pamphlet of education on safety measures prescribed by Myanmar agricultural service (78.5%) respectively. The majority know about pesticides from other sources of information such as from their experience, pesticide labels on containers and pesticide shops and pesticides companies. Regarding to the knowledge of PPE among farm workers in this study is not enough to have chemical safety for them. Thus, further enhancing of education regarding PPE, is suggested for reducing risks of pesticide toxicity among farm workers. To promote safe practice of chemical pesticides, providing knowledge to attain behavioral changes of pesticide users is one of the important factors. It is necessary for health personnel to educate farm workers concerning with pesticide utilization and to promote their knowledge as well. This is because the way of spreading knowledge about safety measures via media is not much effective especially about the use of protective clothes or aprons. It will be more effective if health workers provide knowledge by explaining pros and cons.

4. Organophosphate pesticide affect the male reproductive system by mechanisms such as reduction of sperm density and motility, inhibition of spermatogenesis, reduction of sperm counts, increasing abnormal sperm morphology, change in plasma levels of testosterone, follicle-stimulating hormones (FSH) and Luteinizing hormone (LH). In this study, all the semen quality and serum hormone parameters showed significant differences between growing period and non-growing period. The presence study revealed that there is a positive association between pesticide exposure and male semen quality and serum hormone level between growing period and non-growing period. There is similar to Hossain, F et al., studied that men with occupational pesticide exposures had higher risks for lower semen volume, lower concentration, higher abnormal morphology and decreased sperm motility (98). Also Perez-Herrera indicated that increased serum LH and FSH and decrease Testosterone related with organophosphate exposure (80). Toxic exposures can alter the production, release, or function of hormones that regulate spermatogenesis. Decreases in testosterone can also affect a man's desire for, and ability to perform, sexual intercourse. Some chemical or physical agents can damage a man's testicles, the sperm cells, or the mature sperm. This damage can cause a reduction in the number of sperm produced, a total absence of sperm, changes in their shape or ability to move. Exposures of men to workplace chemicals can also affect reproduction and development if the agent is secreted in the seminal fluid (195). For diagnosis for sperm analysis, (74%) of male ground-nut farmers have become victim of Oligozoospermia which means that below the lower reference limit, in growing period but increased in non-growing period and resulted as (46%) because in non-growing period, the ground-nut farmers have lack of exposure to pesticide residues. Thus, we can draw a generation conclusion that sperm analysis results can be associated with exposure to pesticide residues and time duration. That means the longer the time of exposure to the pesticides, the greater the risk. Some of the other factors include lifestyle, smoking, drinking alcohol, obesity and level of education and proper use of PPE can effect of pesticides on semen analysis and serum hormone levels. This study gave contradictory results, and interpretation was rendered difficult because hormonal values vary according to the time between the last toxicant exposure and the time of hormonal evaluation(201). The hormonal disruption in agricultural workers in the present study, together with results from other experimental

animal studies, suggests that OP exposure disrupts the hypothalamic-pituitary endocrine function and also indicates that FSH and LH are the hormones affected. Finally, the conclusions should promote further evaluation of male reproductive toxicity of commonly used substances or those that are likely to be in contact with human populations, on male fertility and consider that estimations of hormonal parameters are a sensitive marker of pesticide exposure.

5. The average acetyl and plasma cholinesterase levels of ground-nut farmers in growing period were significantly higher than non-growing period and showed that the results tended to chronic exposure with pesticide residues and it might to be chronic health effect problem. Body mass index (BMI) of ground-nut farmers was 18.34 and it was classified in the lower than the normal range (18.5-24.9) by WHO (187). There are suggestions that dietary factors can influence cholinesterase levels. Low levels of cholinesterases have observed in malnutrition (203). However, the findings revealed that ground-nut farmers were exposed to pesticide residues. Sometimes, there was a minor error of temperature range during testing blood period and it might be influenced the performance of the Test-mate cholinesterase kit but average temperature at the tested room in both periods of growing and non-growing was almost the same. Therefore, farm workers with longer than ten years duration of work had more below normal blood cholinesterase level than farm workers with ten years duration. it show that farm workers with more than ten years of work durations needed to monitor their blood cholinesterase level regularly before and after handling or using pesticides. Moreover, they should be avoided handling pesticide if their cholinesterase level is not reach to normal within 14 days.

6. In Myanmar, organophosphate groups, organochlorine groups and carbamate groups are used commonly. But in this study, most of the ground-nut famers used organophosphate group and pyrethroids group. Pesticides are used in combination of at least 2 to 3 and sometimes up to 5 kinds are mixed and sprayed on the crops. These pesticides are used deliberately without any restrictions and precautions and also the empty containers are not disposed-off systemically due to scarcity of the land areas (16). The ubiquitous organophosphates present a continuing health hazard in agricultural, public health eradication programmes and as chemical warfare agents.

Most of the ill-health following exposure to organo-phosphorus compounds has been attributed to the inhibition of cholinesterases (206). Organophosphate pesticides (OP) are suspected of altering reproductive function by reducing brain acetylcholinesterase activity and monoamine levels, thus impairing hypothalamic and/or pituitary endocrine functions and gonadal processes (169). In this study detected some of the symptoms related to nicotinic action of organophosphates on central nervous system and on neuromuscular activity. These symptoms are dizziness, headache, weakness , blurred vision and are of high prevalence, whereas the muscarinic effect such as sweating, hyper salivation and tremors are found and highest in growing period than non-growing period. The most frequent symptoms in growing period were blurred vision (27%), followed by dizziness (13%), headache (12%) and sweating (6%) but all are slightly decrease in non-growing period. The neurotoxic symptoms may be associated with the pesticides' effect on health or may be due to other factors such as heat exhaustion or life styles or socio economic factors which cannot be differentiated in this study. This study could not differentiate the causes of these symptoms suffered by farm workers. However, this study recommended doing an experimental follow-up study to explore the effect of these symptoms. On the other hand, there was no abnormal finding in physical examination. In physical examination, sign and symptoms pesticide toxicity were monitored in all system such as respiratory system, central nervous system, cardiovascular system, etc using physical examination chart and proforma. It included past history of hospitalization and reason for hospitalization. Reporting health symptoms in growing and non-growing period, it was found that there were significant difference in dizziness and blurred vision under central nervous system (p-value <0.05) in this study.

7. In Phase III for dermal exposure assessment, based on the statistical calculation remarking farmers interviews (n=30). The 95^{th} percentile of exposure duration was 19.2 years, exposure frequency was 100.27 days/years, and the body weight was 57.72 kg (at mean). Exposure assessment showed the average daily dose ADD (mean) of the ground-nut farmers at 3.66×10^{-5} mg/kg-day and ADD (RME) was 9.93×10^{-5} mg/kg-day in the growing period among 30 randomly selected samples of the ground-nut farmers. ADD value was huge in this study because duration of exposure was higher. For non-carcinogenic risk characterization, a Hazard Quotient (HQ) was used to

estimate the risk and calculated by this following equation, which was provided from (166). In this study, HQ at mean and 95th percentile RME level were 0.12 and 0.33 and hazard quotient (HQ) of chlorpyrifos for male ground-nut farmers in this area was lower than the acceptable level 1.0 at mean. Therefore this study concluded that they did not get risk from body skin exposure. Exposure may also be increased due to mixing more than one organophosphate pesticide and using more than recommended amount, eating and drinking at the farm areas, and smoking during pesticide application. So that, the suitable of safety risk management should be provide in this area to protect the ground-nut farmers to get risk from pesticide through dermal exposure. Advice and training should be offered on correct pesticide application procedures as well as the right quantities to use in order to minimize risks.

6.2 Limitations

1. The sample size was not small but the generalizability of the findings is restricted because data collection was limited and so that selected workers considered to be representative of all Myanmar agricultural workers.
2. This study was focused only on organophosphate pesticides that use in this community and other groups of pesticides were not investigated.
3. The analysis relied on self-reports of agricultural practices and use of PPE, and as a result there may be inaccurate data. Furthermore, because regulatory practices, and the information levels could change over time, it is possible that safety practices may be affected.
4. Only dermal exposure was done and other sources of exposure may be involved, for example dietary exposure and inhalation route which may be important pathways of exposure.
5. In spite of several studies and laboratory researches, no consistent view exits on the role of chronic pesticide exposure on semen parameters at present study; this is because of the many confounding factors and maybe, it is not logical to compare different studies between developing and developed countries and generalized the conclusions.

6.3 Recommendations

1. Education, training and information activities on pesticide safety should be established and strengthened. Health education should be given to avoid over use of pesticide, not following instruction and to reinforce use of bio-pesticides and adverse effect of pesticide on health and environment especially the organophosphates.
2. Careful and responsible use of pesticide among the farm workers in order to show a consideration for the environment was also needed.
3. This study focused on chlorpyrifos, which was organophosphate pesticides. It was mostly used in this group of pesticide. Therefore, this research studied only chlorpyrifos. Therefore, further study would be required to assess different types of pesticides exposure through multiple routes such as inhalation and dermal routes and also to determine the potential association between pesticide exposure, ChE activity, and health effects.
4. Animal studies proved that it was an association between pesticide exposure with cholesterol level and it can effect the lower level of serum hormonal level. For further study should test upon relation between pesticide exposure with increase cholesterol level and it can effect to serum hormone level in human in agricultural setting.
5. Survey and questionnaire data can be used likely be limited to occupational settings where specific pesticides are known, so that, future in depth research into the relationship between semen quality and exposure to different types of pesticides with a bigger number of participants is recommended so as to facilitate the safe use of pesticides in agricultural practices.
6. This study mainly done in ground-nut farmers who loaded, mixed and sprayed pesticide in the field but we did not focus on local people who lived near the field. The further investigation should survey susceptible group or people who had house near the field.
7. For further studies should have sufficient power and homogenous case groups with an appropriate unexposed control group. Exposure assessment should be made as accurate as possible, preferably confirmed by environmental and/or

biological monitoring and targeted to specific exposures. In addition, end-points should be multiple because toxins act at different sites of the reproductive system.

8. Severity of contamination and level of exposure should be determined as much as possible, preferably confirmed by environmental and biological monitoring.

9. Use of PPE does reduce dermal pesticide exposure but compliance among the majority of occupationally exposed pesticide end users appears to be poor. More research is needed on higher-order controls to reduce pesticide exposure and to understand the reasons for poor compliance with PPE and identify effective training methods.

10. Local responses to the management of pesticides in public health, agriculture and environment sectors are often poor in Myanmar especially in coordination and collaboration may not be sufficiently effective between the principal pesticide regulatory authority (generally the Ministry of Agriculture) and the Ministry of Health, on the evaluation, authorization, monitoring and control of public health pesticides. As a result, not all elements of the pesticide life cycle may be properly regulated and managed. Consequently, problems in pesticide management that could have been recognized and dealt with at an early stage are either overlooked or only materialize in the legislation or enforcement phase. A particular example is the development of insecticide resistance in public health applications which is caused or exacerbated by use of insecticides with the same mode of action in agriculture. The prevention and management of such resistance selection requires routine monitoring of insecticide resistance as well as joint development of a strategy for resistance management between the ministries of health and agriculture.

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APPENDIX

APPENDIX A

Questionnaire

Code …………

Pesticide Exposure and Health Effects Questionnaire

Description

Questionnaire is separtaed into 3 parts: first and second parts consisted of opened and closed questions, the last part has only closed question. The details are showed as following:

Part 1: General Information

The questions ask about subjects' background information including agricultural works and farming descriptions and general health information. There are 16 questionnaires for general information and agricultural works and farming and there are 12 questionnaires for health information and interview by investigator and three trained interviewers by face to face interview method.

Part 2 : Explore knowledge and practice on safe use of pesticide

The information in this part is based on safe use of pesticide utilization by using questionnaires on knowledge and practice on pesticide among ground-nut farmers. There are total 15 knowledge questionnaires and total 20 practice questionnaires and interview by investigator and three trained interviewers by face to face interview method.

Part 3: Favorable Environment

There are 4 questionnaires for asking on available, accessable , affordable upon PPE and getting information about PPE and interview by investigator and three trained interviewers by face to face interview method.

<u>Part 1 : General Information</u>

Code of participant ________________

1. Age________________years

2. Gender ( ) Male ( ) Female

3. Status ( ) Single ( ) Married ( ) Widow ( ) Divorce

4. The highest education level

( ) Uneducated

( ) Primary School

( ) Secondary School

( ) High School

( ) Diploma

( ) Bachelor's Degree or higher

5. Income (ground-nut crop/ year) ------------------------------------kyats/year

6. Smoking

( ) Never

( )Ever

( )Current smoke

- Type ……………………………………..
- No. of cigarette/day ....

7. How many members in your family are ground-nut farmers (including the interviewee)? ____ person (s)

8.If you are married, how many children do you have now? ___________person(s)

<u>Agricultural works and farming descriptions</u>

9. Area cultivated ……………………………………….acres

10. How long do you work in paddy field for one day? _______________ hours/day

11. In one week, how many days do you work in paddy field ? _______________days/week

12. How many years do you apply pesticide in the paddy field? _______________ years

13. In one day, how much do you apply pesticide? _______________ times/day

14. In last year, how many farm areas do you apply pesticide (including your farm and family's farm) ? -----------------------------acres

15. Nowadays, what methods do you use to kill pest in field? (choose more than one choice)

( ) Apply pesticide by yourself

( ) Hire someone to apply pesticide

( ) Invite relative to help

16. Do you do organic culture ?(not use pesticide)

( ) Yes ( ) No

17. Except gound-nut, do you grow other crops?

( ) No ( ) Yes ____

And do you apply pesticide with them?

( ) Yes ( ) No

GENERAL HEALTH INFORMATION

This part interview by researcher and three trained interviewers.

1. Weight_________________kilograms

2. Height______________________centimeters

3. In last crop, do you have any signs or symptoms after inhale or touch pesticide follow this table

Signs and symptoms	Never	Almost Never	During pesticide exposure	Shortly after pesticide application	Suffered when applied after pesticide and so stopped that pesticide
Headache					
Nausea/Vomiting					
Abdomen cramp					
Blurred vision					
Tearing					
Dizziness					
Numbness or pins and needles in					

your hands and feet					
Arms and legs weakness					
Involuntary twitches or jerks in your arms or legs					
Chest tightness					
Difficult breathing					
Infertility					

Part 2 : Explore knowledge and practice on safe use of pesticide

Knowledge Questions

There are total 14 knowledge questionnaires : 7 questionnaires as choice only 1 answer but question 8 to 14 can choose more than one answer.

No	Knowledge Questions		Code
1.	Do pesticide cause dangerous of pet in working places?	( )Yes ( ) No ( ) Do not know	
2.	Do pesticide cause adverse effect on human?	( )Yes ( ) No	

		( ) Do not know	
3.	Is environment damaged by using pesticide?	( )Yes ( ) No ( ) Do not know	
4.	Can pesticide enter the human body accidently?	( )Yes ( ) No ( ) Do not know	
5.	Can pesticide cause toxicity?	( )Yes ( ) No ( ) Do not know	
6.	Can pesticide cause death if pesticide enter the human body accidently?	( )Yes ( ) No ( ) Do not know	
7.	Can you prevent pesticide that enter the body by using PPE?	( )Yes ( ) No ( ) Do not know	

The following questionnaires can choose more than one choice

No	Knowledge questions		Yes	No	Code
8.	Which environmental media can pesticide enter the human body?	1) Water 2) Soil 3) Air			
9.	Which ways can pesticide enter the human body?	1) mouth (ingestion) 2) nose (inhalation) 3) skin (contact)			

10.	Which organs will be damaged by chronic poison of pesticide?	1) Nervous system 2) Respiratory system 3) Hepatic system 4) Reproductive system 5) Renal system 6) Dermal 7) Do not know			
11.	Describe Personal Protective Equipment (PPE) required for using pesticide if you have known them?	1) Hat 2) Goggle/eye cover 3) Mask 4) Standardize Gloves 5) Protective clothes 6) Rubber boot 7) Apron 8) Do not know			
12.	Which behaviors should be avoided during application of pesticide?	1) Eating 2) Smoking 3) Drinking			
13.	Pesticides should not be sprayed in following conditions:	1) Windy condition 2) Under extreme heat of sun			

		3) Raining			
14.	Which site should be started in spraying pesticides?	1) Up wind 2) Down wind 3) Suitable site 4) Do not know			

Practice Questions

There are total 20 practice questionnaires : 4 questionnaires as choice only 1 answer but question 5 to 20 can choose more than one answer.

No.	Practice questionnaires		Code
1.	Do you use registered pesticides in agriculture?	( )Yes ( ) No ( ) Do not know	
2.	Do you read, follow and spray pesticide according to instruction or label?	( )Yes ( ) No ( ) Do not know	
3.	While spraying pesticide, have you use personal protective equipment (PPE)?	( )Yes ( ) No ( ) Do not know	
4.	Do you keep pesticide with food and water?	( )Yes ( ) No ( ) Do not know	

The following questionnaires can choose more than one choice

No	Practice questionnaires		Yes	No	
5.	Where do you store pesticide?	1)Separate-room(separate/high place/locked box) 2) Keep out of children, animals who do not know			

		the hazards of pesticides. 3) Keep out of food and water source 4) None of the above			
6.	Who would you listen to when you decide to purchase pesticide ?	( ) Neighbourhood ( ) Shopkeeper's advice ( ) Advertisment ( ) Agricultural officer ( ) Sales representative			
7.	In general, how do you mix pesticide ?	( )Never follow the bottle instruction label ( ) Follow the bottle instruction label ( ) Follow the neighborhoods' suggestion ( )use more than one type of pesticide			
8.	How do you mix pesticide ?	( ) Wearing rubber gloves and using stirring stick ( ) Wearing fabric gloves and using stirring stick			

		( ) Using hand and using stirring stick ( ) Using hand only			
9	Which personal protective equipment (PPE) do you usually use when you mix pesticide ?	( ) None ( ) Chemical glove ( ) Chemical protective mask ( ) Goggle or glasses ( ) Dust protective mask ( ) Fabric gloves ( ) Normal face mask ( ) Rubber boots ( ) Hat ( ) Apron ( ) fabric ( ) plastic ( ) Clothes coverall			
10.	Mostly, which part of your body contact pesticide when you mix and spray pesticide ?	( ) None ( ) Hands and arms ( ) Face ( ) Body ( ) Legs and foots			
11.	What kind of outfit do you mostly/usually wear when you apply pesticide ?	( ) Short sleeved t-shirt and short pants ( ) Long sleeved t-shirt and long pants ( ) Vest and short pants			

		( ) Vest and long pants ( ) Long sleeved shirt and short pants ( ) Long sleeved shirt and long pants			
12.	If you spill some pesticide on your clothes and body in early morning, when do you change clothes and clean your body?	( ) Change clothes and clean body immediately ( ) Change clothes and clean body after finish work ( ) Change clothes and clean body the end of day			
13.	After you mix and spray pesticide, how do you clean your body?	( ) Wash hands and arms immediately ( ) Wash hands and arms before lunch ( ) Take a bath immediately ( ) Take a bath at noon ( ) Wash hands and arms in evening ( ) Take a bath after finish work			
14.	Which products do you use to clean body after touching	( ) Only water ( ) Water and Soap			

	and mixing pesticide?	( ) Water and Dishwashing			
15.	What do you do with the clothes you wearing after you used pesticide?	( ) Change new clothes immediately ( )Change new clothes after finished spraying ( ) Change new clothes at the end of day ( ) Change new clothes before the start of next day			
16.	How often do you clean your clothes after that clothes contact with pesticide?	( ) Wash it immediately ( ) Keep it and wear it again on next day ( ) Keep it and wear it again whole week ( ) Keep it and wear it again whole month			
17.	What is the method in disposing pesticide container?	( ) Disposing on the ground ( ) Keep to dispose in your landfill ( ) Disposing in the hole ( ) Disposing in nature water source			

		( ) Disposing in garbage ( ) Incinerating			
18.	Do you have lunch in paddy field?	( ) Mostly ( ) Sometime ( ) Often ( ) Never			
19.	Do you smoke eat and drink (water) during spraying pesticide?	( ) Mostly ( ) Sometime ( ) Often ( ) Never			

20. Mostly, which pesticide do you use in your field (Can write more than one choices)

No.	Type of pesticides	Duration (years)	An average usage of pesticide bottles in one year	Reason of choice for pesticide usage			
				Cheap	Easy to purchase	Neighbors' advice	Short time to kill pest

Part 3: Favorable Environment

1. Are Personal Protective Equipment (PPE) in your local area?	1.Yes 2.No	
2. Do you access to buy personal protective equipment (PPE) in your area?	1.Can buy 2.Cannot buy	
3. Can you afford to buy PPE?	1.Can afford 2.Cannot afford	
4. Where do you get information about PPE?	1..Neighbour 2. Sale man 3 .Agricultural sector. 4.Media(Radio/TV/ Newspaper/Journal) 5.Elder 6.Other	

ANNEX (B)

Check List for Agricultural workers

Name of observer-------------------

Code Number -----------------------

Observation will be done by the investigator and three trained interviewer..

Sr.No		Yes	No	Code
1.	Currently use of registered pesticide			
2.	Instruction is included on package			
3.	Do you understand instruction on package of pesticide			
4.	Pesticide is placed safety 1.separate room/high/ in locked room 2.keep with agricultural equipment 3. keep with food			
5.	Water to mix pesticide -rain water -shallow well/tube well -others			
6.	Sufficient amount of water and soap to watch hand and body			
7.	Use of Personal Protective Equipment (PPE)			

APPENDIX (C)

Proforma and Physical Examination Chart

1. Participant code.................................

2. Age ------------------

3. Weight ……………., Height………………….

4. Education

5. Smoke? Yes = 1 , No = 0

6. Eating and / or Drinking During Work: Yes = 1 , No = 0

7. Alcohol: Yes = 1 , No = 0

8. Exposure duration (years)………………

9. Working days/week…………………

10. Working Hours/day………………….

11. Working days/year……………

12. Use of PPE: Yes = 0, No = 1

13. Washing hands after working : Yes = 0, No = 1

Past History of Hospitalization: Yes = 1 , No = 0

14. Hematological

15. Cardiovascular

16. Neurological

17. Musculoskeletal

18. Gastrointestinal

19. Other

If other, specify ....

General Symptoms: Yes = 1 , No = 0

20. Weakness

21. Itching of skin

22. Sweating

Gastrointestinal: Yes = 1 , No = 0

23. Nausea

24. Vomiting

25. Pain in abdomen

26. Loose motion

Central Nervous System: Yes = 1 , No = 0

27. Headache

28. Dizziness

29. Irritability

30. Paraesthesia

31. Blurred vision

32. Mental confusion

33. Convulsion

34. Hallycination

35. Unconsciousness

Physical Signs: Yes = 1 , No = 0

36. Pupilalry constriction

37. Conjunctival redness

38. Pallor

39. Cyanosis

40. Hyperpyrexia

Cardiovascular System

41. Ausculation Normal = 1, Abnormal = 0

If abnormal, specify ----------------

Respiratory System

42. Auscultation Normal = 1 , Abnormal = 0

If abnormal, describe **………….**

43. Respiratory depression Yes = 1 , No = 0

44. Pulmonary oedema Yes = 1 , No = 0

Blood Pressure

45. Systolic --------------mm hg

46. Diastolic -------------mm hg

Gastrointestinal Tract

47. Liver NP = 1 , P = 0

48. Spleen : NP = 1 , P = 0

Skin: Yes = 1 , No = 0

49. Redness

50. Swelling

51. Dermatitis

52. Others, If others specify ---

Central Nervous System

53. Muscle bulk : Normal = 1 , Hypertrophy = 0 , Wasting = 0

54. Fasciculation: Present = 1 , Absent = 0

55. Tremours : Normal = 1 , Decreased = 0

56. Lower limb: Normal = 1 , Decreased = 0

Tone

57. Upper limb: Normal = 1 , Increased = 0 , Decreased = 0

58. Lower limb: Normal = 1 , Increased = 0 , Decreased = 0

Tendon reflexes

59. Loss of biceps jerk : Normal = 1 , Decreased = 0

60. Loss of triceps jerk : Normal = 1 , Decreased = 0

61. Loss of supinator jerk : Normal = 1 , Decreased = 0

Lower Limbs

62. Loss of ankle jerk : Normal = 1 , Decreased = 0

63. Loss of knee jerk : Normal = 1 , Decreased = 0

Reproductive System Yes = 1 , No = 0

64. Infertility

Biochemical Test

65. Seminal Analysis : Normal = 1, Below normal = 0

66. Serum Hormones : : Normal = 1, Below normal = 0

67. Blood cholinesterase: Normal = 1, Below normal = 0

68. For Stress Test,

For each question, choose the best answer for how you left over the past week.

No.	Questions	Yes	No	Code
1.	Are you basically satisfied with your life?			
2.	Have you dropped many of your activities and interests?			
3.	Do you feel that your life is empty?			
4.	Do you often get bored?			

5. Are you in good spirits most of the time?
6. Are you afraid that something bad is going to happen to you ?
7. Do you feel happy most of time?
8. Do you often feel helpless?
9. Do you prefer to stay at home, rather than going out and doing new things?
10. Do you feel you have more problems with memory than most?
11. Do you think it is wonderful to be alive now?
12. Do you feel pretty worthless the way you are now?
13. Do you feel full of energy?
14. Do you feel that your saturation is hopeless?
15. Do you think that most people are better off than you are?

The scale is scored as follows: 1 point for each response in capital letters. A score of 0 to 5 is normal; a score above 5 suggests depression and have stress.

In this study, stress test results are as follows:

Stress test	Growing Period		Non-Growing Period	
	Frequency	Percentage	Frequency	Percentage
Normal (score 0 to5)	97	97	99	99
Abnormal (score above 5)	3	3	1	1

APPENDIX (D)

Scoring System

Knowledge Questions Scoring

Correct answer score – 1 Not correct answer score - 0

Knowledge Questions

There are total 14 knowledge questionnaires : 7 questionnaires as choice only 1 answer but question 8 to 14 can choose more than one answer.

No	Knowledge Questions		Scoring
1.	Do pesticide cause dangerous of pet in working places?	( )Yes ( ) No ( ) Do not know	1 0 0
2.	Do pesticide cause adverse effect on human?	( )Yes ( ) No ( ) Do not know	1 0 0
3.	Is environment damaged by using pesticide?	( )Yes ( ) No ( ) Do not know	1 0 0
4.	Can pesticide enter the human body accidently?	( )Yes ( ) No ( ) Do not know	1 0 0
5.	Can pesticide cause toxicity?	( )Yes ( ) No ( ) Do not know	1 0 0
6.	Can pesticide cause death if pesticide enter the human body accidently?	( )Yes ( ) No ( ) Do not know	1 0 0
7.	Can you prevent pesticide that enter the body by using PPE?	( )Yes ( ) No ( ) Do not know	1 0 0

The following questionnaires can choose more than one choice

No	Knowledge questions		Yes	No	Scoring
9.	Which environmental media can pesticide enter the human body?	1) Water 2) 2) Soil 3) Air	1 1 1	0 0 0	1 1 1
10.	Which ways can pesticide enter the human body?	1) mouth (ingestion) 2) nose (inhalation) 3) skin (contact)	1 1 1	0 0 0	1 1 1
11.	Which organs will be damaged by chronic poison of pesticide?	1) Nervous system 2) Respiratory system 3) Hepatic system 4) Reproductive system 5) Renal system	1 1 1 1 1	0 0 0 0 0	1 1 1 1 1
12.	Describe Personal Protective Equipment (PPE) required for using pesticide if you have known them?	1) Hat 2) Mask 3) Standardize Gloves	1 1 1	0 0 0	1 1 1

		4) Protective clothes 5) Rubber boot 6) Apron	1 1 1	0 0 0	1 1 1
13.	Which behaviors should be avoided during application of pesticide?	1) Eating 2) Smoking 3) Drinking	1 1 1	0 0 0	1 1 1
14.	Pesticides should not be sprayed in following conditions:	1) Windy condition 2) Under extreme heat of sun 3) Raining	1 1 1	0 0 0	1 1 1
15.	Which site should be started in spraying pesticides?	1) Up wind 2) Down wind 3) Suitable site 4) Do not know	1 0 0 0	0 0 0 0	1 0 0 0

Knowledge grading using 15 questions. A correct answer will give 1 score and 0 score for wrong answer. The scores vary from 0-31 points and will classify into 3 levels as follows: Bloom's cut off point, 60%-80%.

Scores	Descriptions
0-10 (less than 60%)	Low levels
11-20 (60-79%)	Moderate levels
21-31 (≥80%)	High levels

Practice Questions

There are total 20 practice questionnaires : 4 questionnaires as choice only 1 answer but question 5 to 20 can choose more than one answer.

No.	Practice questionnaires		Scoring
1.	Do you use registered pesticides in agriculture?	( )Yes ( ) No ( ) Do not know	1 0 0
2.	Do you read, follow and spray pesticide according to instruction or label?	( )Yes ( ) No ( ) Do not know	1 0 0
3.	While spraying pesticide, have you use personal protective equipment (PPE)?	( )Yes ( ) No ( ) Do not know	1 0 0
4.	Do you keep pesticide with food and water?	( )Yes ( ) No ( ) Do not know	1 0 0

The following questionnaires can choose more than one choice

No	Practice questionnaires		Yes	No	Score
5.	Where do you store pesticide?	1)Separate-room(separate/high place/locked box)	1	0	1
		2) Keep out of children, animals who do not know the hazards of pesticides.	1	0	1
		3) Keep out of food and water source	1	0	1
		4) None	0	0	0
6.	Who would you listen to when you decide to purchase pesticide ?	( ) Neighbourhood	1	0	1
		( ) Shopkeeper’s advice	1	0	1
		( ) Advertisment	1	0	1

		( ) Agricultural officer	1	0	1
		( ) Sales representative	1	0	1
7.	In general, how do you mix pesticide ?	( )Never follow the bottle instruction label	0	0	0
		( ) Follow the bottle instruction label	1	0	1
		( ) Follow the neighborhoods' suggestion	0	0	0
		( )use more than one type of pesticide	0	0	0
8.	How do you mix pesticide ?	( ) Wearing rubber gloves and using stirring stick	1	0	1
		( ) Wearing fabric gloves and using stirring stick	0	0	0
		( ) Using hand and using stirring stick	0	0	0
		( ) Using hand only	0	0	0
9	Which personal protective equipment (PPE) do you usually use when you mix pesticide ?	( ) None	0	0	0
		( ) Chemical glove	0	0	0
		( ) Chemical protective mask	1	0	1
		( ) Goggle or glasses	1	0	1
		( ) Dust protective mask	0	0	0
		( ) Fabric gloves	0	0	0
		( ) Normal face mask	0	0	0
		( ) Rubber boots	1	0	1
		( ) Hat	1	0	1
		( ) Apron	1	0	1
		( ) fabric	0	0	0
		( ) plastic	0	0	0
		( ) Clothes coverall	0	0	0

10.	Mostly, which part of your body contact pesticide when you mix and spray pesticide?	( ) None	0	0	0
		( ) Hands and arms	1	0	1
		( ) Face	1	0	1
		( ) Body	1	0	1
		( ) Legs and foots	1	0	1
11.	What kind of outfit do you wear when you apply pesticide?	( ) Short sleeved t-shirt and short pants	0	0	0
		( ) Long sleeved t-shirt and long pants	0	0	0
		( ) Vest and short pants	0	0	0
		( ) Vest and long pants	0	0	0
		( ) Long sleeved shirt and short pants	0	0	0
		( ) Long sleeved shirt and long pants	1	0	1
12.	If you spill some pesticide on your clothes and body in early morning, when do you change clothes and clean your body?	( ) Change clothes and clean body immediately	1	0	1
		( ) Change clothes and clean body after finish work	0	0	0
		( ) Change clothes and clean body at noon	0	0	0
		( ) Change clothes and clean body the end of day	0	0	0
13.	After you mix and spray pesticide, how do you clean your body?	( ) Wash hands and arms immediately	1	0	1
		( ) Wash hands and arms before lunch	0	0	0
		( ) Take a bath immediately	1	0	1
		( ) Take a bath at noon	0	0	0

		( ) Wash hands and arms in evening ( ) Take a bath after finish work	0 1	0 0	0 1
14.	Which products do you use to clean body after touching and mixing pesticide?	( ) Only water ( ) Water and Soap ( ) Water and Detergent ( ) Water and Dishwashing	0 1 0 0	0 0 0 0	0 1 0 0
15.	What do you do with the clothes you wearing after you used pesticide?	( ) Change new clothes immediately ( )Change new clothes after finished spraying ( ) Change new clothes at the end of day ( ) Change new clothes before the start of next day	1 0 0 0	0 0 0 0	1 0 0 0
16.	How often do you clean your clothes after that clothes contact with pesticide?	( ) Wash it immediately ( ) Keep it and wear it again on next day ( ) Keep it and wear it again whole week ( ) Keep it and wear it again whole month	1 0 0 0	0 0 0 0	1 0 0 0

17.	What is the method in disposing pesticide container?	( ) Disposing on the ground	0	0	0
		( ) Keep to dispose in your	0	0	0
		landfill			0
		( ) Disposing in the hole	0	0	0
		( ) Disposing in nature water	0	0	
		source			0
		( ) Disposing in garbage	0	0	1
		( ) Incinerating	1	0	
18.	Do you have lunch in paddy field?	( ) Mostly	0	0	0
		( ) Sometime	0	0	0
		( ) Often	0	0	0
		( ) Never	1	0	1
19.	Do you smoke eat and drink (water) during spraying pesticide?	( ) Mostly	0	0	0
		( ) Sometime	0	0	0
		( ) Often	0	0	0
		( ) Never	1	0	1

20. Mostly, which pesticide do you use in your field (Can write more than one choices)

No.	Type of pesticides	Duration (years)	An average usage of pesticide bottles in one year	Reason of choice for pesticide usage			
				Cheap	Easy to purchase	Neighbors' advice	Short time to kill pest

Practice scores range from 0 to 34 and will be classified into 3 levels as Boom's Cut of point..

Good practice	27-34 scores (≥80%)
Fair practice	16-26 scores (60%-79%)
Poor practice	0-15 scores (less than 60%)

APPENDIX (E)

Ethics Review Committee

Department of Medical Research

Ministry of Health

Republic of the Union of Myanmar

Informed Consent Form for Clinical Trial

This informed consent form is for Pesticides exposure and health risk assessment: A case study of health effect on male farmer's reproductive system in KyaukKan village of Nyaung-U District, Mandalay Region, Myanmarand who we are inviting to participate in research.

Name of Principal Investigator – Dr. Thant ZawLwin

Name of Organization – College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand.

Name of Proposal – Pesticides exposure and health risk assessment: A case study of health effect on male farmer's reproductive system in KyaukKan village of Nyaung-U District, Mandalay Region, Myanmar

PART I: Information Sheet

Introduction

(1) Introduction

I am Dr Thant ZawLwin, and learning for PhD of Public Health in College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand.. I am doing research on Pesticides exposure and health risk assessment: A case study of health effect on male farmer's reproductive system in KyaukKan village of Nyaung-U District, Mandalay Region, Myanmar. I am going to give you information and invite you to be part of this research. Before you decide, you can talk to anyone you feel comfortable with about the research. This consent form may contain words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me or of another researcher.

(2) Purpose of the research

Purpose of the research is Pesticides exposure and health risk assessment: A case study of health effect on male farmer’s reproductive system in KyaukKan village of Nyaung-U District, Mandalay Region, Myanmar

(3) Type of Research Intervention

This research will involve your participation by answering the questionnaires. by doing physical examination , skin exposure test by using patch samples and by testing of level of cholinesterase enzyme, hormonal levels in whole blood and sperm analysis to find out the relationship with pesticides.

(4) Participant Selection

You are being invited to take part in this research because we feel that you have knowledge on pesticides and handling or using pesticides in your work.

(5) Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. If you choose not to participate, all the services you receive at the unit will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier.

(6) Procedures and Protocol

I am inviting you to take part in this research project. If you accept, you will be answered the survey questionnaire. If you do not wish to answer any of the questions, you will move on to the next question. No one else but the interviewer will be present unless you would like someone else to be there. The information recorded is confidential, and no one else except Dr Thant ZawLwin will access to the information documented during your interview. After that, you will be examined by the researcher using proforma for physical examination and again assessed by using questionnaire. Then, your blood sample will be taken for testing serum hormonal level and cholinesterase enzyme in whole blood, semen analysis and do not include any pharmacological therapy. Some individuals may suffer from pain and swelling due to the needle injection. In these circumstances, that individual will be treated immediately by giving effective treatments. If necessary, vegetables or fruit or water or soil sample from your farm will be taken to test pesticides residue.

(7) Benefits

There will be no direct benefit to you, but your participation is likely to help us to pesticides exposure and health risk assessment: A case study of health effect on male farmer's reproductive system in KyaukKan village of Nyaung-U District, Mandalay Region, Myanmar

(8) Incentives

You will not be provided any incentive to take part in the research.

(9) Confidentiality

The research being done in the community may draw attention and if you participate you may be asked questions by other people in the community. We will not be sharing information about you to anyone outside of the research team. The information that we collect from this research project will be kept private. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will keep the number safely.

(10) Sharing the Results

The knowledge that we get from this research will be shared with you and local health authorities before it is made widely available to the public. We will publish the results so that other interested people may learn from the research.

(11) Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so, and choosing not to participate will not affect your rights and advantages in any way.

(12) Who to Contact

If you have any questions, you can ask them now or later. If you wish to ask questions later, you may contact Dr Thant ZawLwin, phone number. 09252285453 (Myanmar Number).

This proposal has been reviewed and approved by the Institutional Ethical Review Committee, Department of Medical Research (Lower Myanmar), Ministry of Health, which is a committee whose task is to make sure that research participants are protected from harm. If you wish to find out more about the committee, contact the secretary of committee at the Department of Medical Research (Lower Myanmar), No (5), Ziwaka Road, Dagon Township, Yangon, Phone 01-375447 ext.118.

APPENDIX (F)

Body Surface Area

Table F Equation parameters for calculating adult body surface area

Body Part	N	Equation for surface areas (m^2)			P	R^2	S.E.
		a_0	W^{a1}	H^{a2}			
Head							
Female	57	0.0256	0.124	0.189	0.01	0.302	0.00678
Male	32	0.0492	0.339	-0.0950	0.01	0.222	0.0202
Trunk							
Female	57	0.188	0.647	-0.304	0.001	0.877	0.00567
Male	32	0.0240	0.808	-0.0131	0.001	0.894	0.0118
Upper Extremities							
Female	57	0.0288	0.341	0.175	0.001	0.526	0.00833
Male	48	0.00329	0.466	0.524	0.001	0.821	0.0101
Arms							
Female	13	0.00223	0.201	0.748	0.01	0.731	0.00996
Male	32	0.00111	0.616	0.561	0.001	0.892	0.0177
Upper Arms							
Male	6	8.70	0.741	-1.40	0.25	0.576	0.0387
Forearms							
Male	6	0.326	0.858	-0.895	0.05	0.897	0.0207
Hands							
Female	12[b]	0.0131	0.412	0.0274	0.1	0.447	0.0172
Male	32	0.0257	0.573	-0.218	0.001	0.575	0.0187
Lower Extremities[c]	105	0.00286	0.458	0.696	0.001	0.802	0.00633
Legs	45	0.00240	0.542	0.626	0.001	0.780	0.0130
Thighs	45	0.00352	0.629	0.379	0.001	0.739	0.0149
Lower legs	45	0.000276	0.416	0.973	0.001	0.727	0.0149
Feet	45	0.000618	0.372	0.725	0.001	0.651	0.0147

[a] $SA = a_0 W^{a1} H^{a2}$
W = Weight in kilograms; H = Height in centimeters; P = Level of significance; R^2 = Coefficient of determination;
SA = Surface Area; S.E. = Standard error; N = Number of observations
[b] One observation for a female whose body weight exceeded the 95 percentile was not used.
[c] Although two separate regressions were marginally indicated by the F test, pooling was done for consistency with individual components of lower extremities.
Source: U.S. EPA, 1985.

Adapted from: US EPA, 1997

APPENDIX (G)

Risk Communication Material

In this study, the development of risk communication materials was hand books with communicated using PPE picture for encouraging ground-nut farmers to realize and concern their health.

အဝတ်အစားကုန်ကျစားရိတ်

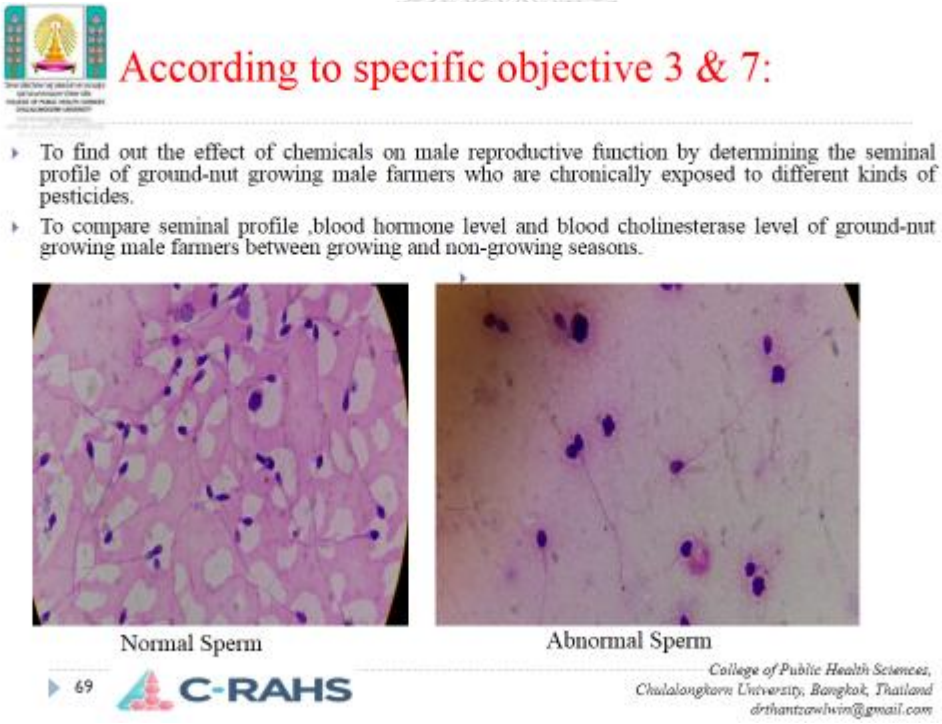
ဦးထုပ် ၃၅၀၀
မျက်မှန် ၁၀၀၀
နှာခေါင်းစည်း ၅၀၀
ဝတ်စုံ ၅၈၅၀
လက်အိတ် ၆၀၀
ဖိနပ် ၂၆၀၀
စုစုပေါင်း ၁၄၀၅၀ကျပ်

အဝတ်အစားကုန်ကျစားရိတ်

ဦးထုပ် ၃၅၀၀
မျက်မှန် ၁၀၀၀
နှာခေါင်းစည်း ၅၀၀
ဝတ်စုံ ၂၀၀၀
လက်အိတ် ၆၀၀
ဖိနပ် ၂၆၀၀
စုစုပေါင်း ၁၀၂၀၀ ကျပ်

APPENDIX (H)

Observation Check for storage and related photos



According to specific objective 4 & 7:

- To determine the blood hormonal level of ground-nut growing male farmers for finding out the effect of chemicals on male reproductive function who are chronically exposed to different kinds of pesticides.
- To compare seminal profile ,blood hormone level and blood cholinesterase level of ground-nut growing male farmers between growing and non-growing seasons.

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According to specific objective 5 & 7:

- To measure the biomarkers of pesticide exposure among the farmers by blood cholinesterase monitoring in ground-nut farmers.
- To compare seminal profile ,blood hormone level and blood cholinesterase level of ground-nut growing male farmers between growing and non-growing seasons.

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According to specific objective 6:

- To assess the risk related to dermal exposure by using gauze patch samples in ground-nut farmers.

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Health Education and Demonstration PPE

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Hand Washing Practice together with Myanmar Actor and Participants

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Research Group with participants

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APPENDIX (H)

Normality Test

1. Normality test for Blood Cholinesterase test in both growing period and non-growing period

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Ache U/ml	.118	100	.002	.948	100	.001
Q u/g	.087	100	.057	.980	100	.130
Pche u/ml	.071	100	.200*	.978	100	.086
Ache U/ml	.085	100	.069	.974	100	.046
Q u/g	.084	100	.079	.973	100	.036
Pche u/ml	.073	100	.200*	.976	100	.062

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

2. Normality test for Blood Hormone Level (FSH, LH, Testosterone) in both growing period and non-growing period

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	Df	Sig.
FSH Growing Period	.180	100	.000	.710	100	.000
LH Growing Period	.098	100	.018	.930	100	.000
Testosterone Growing Period	.077	100	.148	.961	100	.005
FSH Non-Growing Period	.162	100	.000	.792	100	.000
LH Non-Growing Period	.122	100	.001	.904	100	.000
Testosterone Non-Growing Period	.105	100	.009	.935	100	.000

a. Lilliefors Significance Correction

3. Normality test for Seminal analysis (Sperm volume, $_{p}$H, Viscosity, Motility, Morphology, Sperm count) in both growing period and non-growing period

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	Df	Sig.
Sperm Volume Growing Period	.130	100	.000	.930	100	.000
Ph of the specimen Growing Period	.257	100	.000	.854	100	.000
Viscosity Growing Period	.242	100	.000	.854	100	.000
Motility Growing Period	.144	100	.000	.929	100	.000
Morphology Growing Period	.150	100	.000	.948	100	.001
Sperm count Growing Period	.321	100	.000	.344	100	.000
Sperm Volume Non-Growing Period	.164	100	.000	.897	100	.000
pH Non-Growing Period	.295	100	.000	.774	100	.000
Viscosity Non-Growing Period	.224	100	.000	.798	100	.000
Motility Non-Growing Period	.223	100	.000	.891	100	.000
Morphology Non-Growing Period	.203	100	.000	.933	100	.000
Sperm count	.215	100	.000	.822	100	.000

a. Lilliefors Significance Correction

WHO BMI Range

WHO BMI Range

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Moderate underweight (16.0-16.9 kg/m2)	2	2.0	2.0	2.0
	Mild underweight (17.0-18.49 kg/m2)	76	76.0	76.0	78.0
	Normal weight (18.5-24.9 kg/m2)	18	18.0	18.0	96.0
	Preobese (25-29.9 kg/m2)	4	4.0	4.0	100.0
	Total	100	100.0	100.0	

VITA

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WORK EXPERIENCES

I got my M.B.,B.S. degree in December 2005, From that time; I performed curative and preventive measures regarding both non-communicable Diseases and communicable Diseases. I got my master degree, M.Med.Sc (Public Health), in Dec 2008. And I was posted as Assistant Lecturer of Defence Services Medical Academy. I performed teaching activities on both undergraduate and postgradute candidates concerning public health. I joined rescue team for victims during cyclone Nargis in May, 2008. I got Master of Primary Health Care Management(MPHM) from Mahidol University, Thailand in 2011. I involved in prevention and control of diseases, outbreak investigation and also in teaching on undergraduate as well as postgraduate candidates. I helped in many dissertations and thesis on statistical procedures, research methodology and referencing format. And I worked as public health officer at Department of Finance and Procurement in Directorate of Medical Services upto 13 May 2013 and now I am working again as Assistant Lecturer in Department of Public Health in Defence Services Medical Academy. Currently I am studying as PhD student in College of Public Health Sciences, Chulalongkorn University especially studying in Environmental and Occupational Health.

FIELD OF SPECIALTY/RESEARCH INTEREST

Environmental Health and Occupational Health, Health Care Management, Biostatistics, Epidemiology, Project Evaluation, Quantitative and Qualitative Research, Health Economic,