GREEN TOBACCO SICKNESS (GTS) IN THAI TRADITIONAL TOBACCO FARMERS RELATED TO THEIR OCCUPATIONAL EXPOSURE IN NAN PROVINCE, THAILAND

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ธนูศิลป์ สลีอ่อน : กลุ่มอาการป่วยจากการสัมผัสใบยาสูบของเกษตรกรผู้ปลูกยาสูบพันธุ์พื้นเมืองในจังหวัดน่าน ประเทศไทย (GREEN TOBACCO SICKNESS (GTS) IN THAI TRADITIONAL TOBACCO FARMERS RELATED TO THEIR OCCUPATIONAL EXPOSURE IN NAN PROVINCE, THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. วัฒน์สิทธิ์ ศิริวงศ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร. Mark G. Robson, 164 หน้า.

การศึกษาวิจัยนี้ มีวัตถุประสงค์เพื่อประเมินความชุกของกลุ่มอาการป่วยจากการสัมผัสใบยาสูบของเกษตรกรผู้ปลูกยาสูบพันธุ์พื้นเมืองในจังหวัดน่าน ประเทศไทย และการวัดระดับนิโคตินโดยใช้อนุพันธ์โคตินินในน้ำลายและการวัดระดับการสัมผัสกับสารเคมีกำจัดศัตรูพืชในเลือดและน้ำเหลืองโดยชุดทดสอบ Test Mate OP Kit พฤติกรรมการป้องกันตนเองของเกษตรกรและการหาความสัมพันธ์ระหว่างความชุกของกลุ่มอาการป่วยกับระดับโคตินินในน้ำลาย ระดับการสัมผัสสารเคมีกำจัดศัตรูพืชและพฤติกรรมการป้องกันตนเองของเกษตรกรตำบลพระพุทธบาท อำเภอเชียงกลาง และตำบลผาตอ อำเภอท่าวังผา จังหวัดน่าน เป็นการศึกษาแบบภาคตัดขวางชนิดวิเคราะห์ กลุ่มตัวอย่างคือตัวแทนของครัวเรือนเกษตรกรผู้ปลูกยาสูบพันธุ์พื้นเมือง จำนวน 473 คน จากตำบลพระพุทธบาท319 คนและตำบลผาตอ 154คน โดยวิธีการสุ่มตัวอย่างแบบมีระบบและเก็บข้อมูลด้วยวิธีการสัมภาษณ์แบบตัวต่อตัวตามแบบสอบถามที่สร้างขึ้น วิเคราะห์ข้อมูลโดยใช้สถิติเชิงพรรณนา และสถิติวิเคราะห์การถดถอยโลจิสต์ติก การทดสอบค่าไคว์-สแควร์ สัมประสิทธิ์สหสัมพันธ์ของสเพียร์แมน ผลการศึกษาพบว่า ความชุกของการเกิดกลุ่มอาการป่วย(GTS)จากการสัมผัสใบยาสูบของเกษตรกรผู้ปลูกยาสูบพันธุ์พื้นเมืองในจังหวัดน่านเท่ากับร้อยละ 22.62 มีปัจจัยที่เกี่ยวข้องอย่างมีนัยสำคัญทางสถิติได้แก่ เพศ การสูบบุหรี่ ผื่นที่ผิวหนัง การสวมเสื้อผ้าเปียกชื้น ขั้นตอนการบ่มใบยาสูบ การรดน้ำต้นยาสูบ ส่วนการสวมถุงมือยางไม่มีความแตกต่างของการเจ็บป่วย (Chi-square test, $p > 0.05$) การสัมผัสสารเคมีกำจัดศัตรูพืชพบระดับเสี่ยงในเม็ดเลือด(AChE) ร้อยละ 61.90 และระดับเสี่ยงในน้ำเหลือง(PChE) ร้อยละ 42.86 และระดับปลอดภัยในเม็ดเลือด(AChE) ร้อยละ 38.10 และระดับปลอดภัยในน้ำเหลือง (PChE) ร้อยละ 57.14 ซึ่งอาจสนับสนุนให้เกิดกลุ่มอาการเจ็บป่วย(GTS)ได้โดยสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติ (Chi-square test, $p<0.05$) การทดสอบระดับนิโคตินในน้ำลายของเกษตรกรผู้ปลูกยาสูบและตรวจซ้ำ7 ครั้ง พบว่าระดับนิโคตินมีความสัมพันธ์กับการเกิดกลุ่มอาการเจ็บป่วย(GTS)ในทุกครั้งที่ทดสอบ และการทดสอบครั้งที่ 6 (T6) พบระดับความสัมพันธ์สูงสุดระหว่างระดับนิโคตินในน้ำลายกับการเจ็บป่วย (Spearman's correlation coefficient=0.735, $P<0.01$) และพบในกระบวนการทำยาสูบแห้งหรือยาเส้นด้วยซึ่งแตกต่างจากการศึกษาครั้งก่อนที่พบในขณะเก็บใบยาสูบดิบ การเจ็บป่วย(GTS)และระดับนิโคตินในน้ำลายมีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับการใส่หน้ากากอนามัย และการเปลี่ยนเสื้อผ้าที่เปียกในการรดน้ำต้นยาสูบ (Spearman's correlation coefficient=0.894, 0.496, $P<0.01$) ตามลำดับ แนะนำให้มีการให้โปรแกรมสุขศึกษาเพื่อเพิ่มความตระหนักถึงอันตรายและการป้องกันตนเองในเกษตรกรและการศึกษาผลกระทบในระยะยาว

สาขาวิชา สาธารณสุขศาสตร์	ลายมือชื่อนิสิต ....
ปีการศึกษา 2557	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
	ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

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THANUSIN SALEEON: GREEN TOBACCO SICKNESS (GTS) IN THAI TRADITIONAL TOBACCO FARMERS RELATED TO THEIR OCCUPATIONAL EXPOSURE IN NAN PROVINCE, THAILAND. ADVISOR: ASSOC. PROF. WATTASIT SIRIWONG, Ph.D., CO-ADVISOR: PROF. DR. MARK G. ROBSON, Ph.D.,MPH,DrPH, 164 pp.

The aim of this study was to investigate prevalence of Green Tobacco Sickness (GTS) and occupational exposure on Thai traditional tobacco farmers in Nan Province, Thailand. To measure salivary cotinine levels and to measure pesticide exposure levels by Test Mate OP Kit and personal protective behaviors on tobacco farmers. To determine the relationship between GTS and salivary cotinine levels, pesticide exposure levels, personal protective behaviors. This study was a cross-sectional study and prospective study that conducted on 473 tobacco farmers; 319 Thai traditional tobacco farmers from Praputtabath Sub-District, Chiangklang District and 154 from Phatow Sub-district in Thawangpha District were randomly selected and interviewed in person by means of questionnaires and environmental surveying. Descriptive statistics including Chi-squared Test, spearman's correlation (Spearman's rho) and multiple logistic regression analysis were used to potentially identify risk factors pertaining to GTS. The prevalence of GTS was found to be 22.62%. risk factors which are associated with GTS were dependent of certain farmer characteristics; gender, smoking, skin rash, wearing wet suit, process of curing tobacco leaves and watering tobacco plants. Almost of them were using rubber latex gloves that it is possibility to increase nicotine absorption due to climate weather may promote sweat on their hand and were not statistically significantly associated with GTS (Chi-square test, $p>0.05$). The prevalence of risky level of AChE was 61.90% and safe level was 38.10%, risk level of PChE was 42.86% and safe level was 57.14%. However, pesticide was not applied in all period and a symptoms of GTS in which some of them do not use pesticide before, thus possible to indicate a safe level of AChE and PChE contribute to associate with nicotine poisoning in the other name of GTS(Chi-square test, $p<0.05$) .From this study demonstrated the usefulness to use salivary cotinine level measured by NicAlert™ cotinine test strips (NCTS), were well correlated with farmers who working with dry tobacco producing. Salivary cotinine levels were also significantly correlated with the prevalence of GTS among tobacco farmers group in any time to testing across crop season.The six test (T6) was strongly correlate between salivary cotinine levels and GTS (Spearman's correlation coefficient=0.735, $P<0.01$) Dealing with strong positive correlated between wore mask, good practices through changing wet suit during work and GTS is most remarkable from this study(Spearman's correlation coefficient=0.894, 0.496, $P<0.01$) respectively. Finally, the long-term effects of such exposure should be investigated and health education program with health risk exposure for increase awareness of farmers is recommended.

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

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CHAPTER I INTRODUCTION

1.1Background and Rationale

Nicotiana tabacum L. is grown in over 100 countries. The largest plantations are located in China, Brazil, India, the United States, and Malawi; these countries together account for two-thirds of the world's tobacco production (1). Tobacco farming is associated with the hazard of green tobacco sickness henceforth GTS. The disease originates from nicotine's ability to penetrate through the skin of workers who cultivate and harvest tobacco(1), (2), (3); GTS is an occupational illness reported by tobacco workers worldwide(4), (5), (6), (7). Previous studies have shown that dermal absorption of nicotine from plant surfaces gives rise to characteristic GTS symptoms(8), (9), (10). GTS morbidity concerns nearly a quarter of tobacco workers, with typical symptoms including nausea, vomiting, headache, abdominal cramps, breathing difficulty, abnormal body temperature, pallor, chills, fluctuations in blood pressure and heart rate, drenching sweats, and increased salivation(1), (2), (3). In the North of Thailand, Nan Province is one of the most famous areas well known for cultivation of traditional Thai tobacco plants. Traditional Thai Tobacco (*Nicotiana tabacum* L.) is known as a non-Virginia type tobacco. Its mature leaves are thicker and contain three to four times more nicotine than the leaves of a Virginia type tobacco. Table 1, from 2012 to 2013, the traditional tobacco cultivation areas increased by 50% due to favorable prices and an increase in profits which encouraged farmers to cultivate more. Table 2, the provincial total production of the tobacco plant was around 3.7 Million kilograms from a total area of 7,190 *Rais* (1 Acre = 2.539 *Rais*). The largest tobacco harvest was reported in 2013 from Thawangpha District, approximately 1.6 million kilograms; Chiangklang District, about 920,000 kilograms; and Pua District, around 715,300 kilograms, respectively.

Table 1 Traditional Tobacco Cultivation area and production in Nan Province by seasonal from 2009-2012.

Season	Area for Cultivation (*Rais*)	Production (Kg)	Production (Kg/*Rai*)
2009/2010	4,900	7,840,000	1,600
2010/2011	4,802	7,731,220	1,610
2011/2012	7,190	3,763,900	522

Table 2 Traditional Tobacco Cultivation area and production in Nan Province by District season 2011/2012.

District	Households	Area for cultivation (*Rai*)	Product(Kg)	Product average (Kg/*Rai*)
Thawangpha	954	3,411	1,604,600	470
Chiangklang	730	2,963	920,000	310
Pua	191	688	715,300	1,109
Santisuk	50	108	459,000	3,000
Muang Nan	5	20	65,000	1,857
Sum	**1,930**	**7,190**	**3,763,900**	**522**

Traditional tobacco cultivation and dry tobacco production in Thailand differ from those found in western countries. Cultivation is a continuous process to maintain the tobacco plants, especially watering activities with which farmers may come into contact with wet tobacco leaves. Almost all farmers water their plants in the morning or in the evening. The process of Thai traditional tobacco production involves seeding, cultivating, and transplanting to the tobacco field; maintaining tobacco plants with fertilizer and pesticide applications; removing axillaries buds; cutting the top of tobacco plants; and removing weeds from the field. After 100 to 120 days, tobacco leaves can be picked and then can be transferred for subsequent processing. Tobacco leaves are cured until ripe, and then the stems are removed by drawing and rolling tobacco leaves in a bundle so that it can be prepared for slicing by the cutting machine. The sliced tobacco leaves are brought to a bamboo rack, shredded, and then left to dry in a dry rack in direct sunlight for one to three days. Every day and night, the farmers must reverse the bamboo rack so as to control the color of the tobacco line which can be adjusted by spraying a dry tobacco extract in the evening. The nighttime dew will soften the tobacco slices and allow them to be folded for packaging, with ten kilograms of tobacco slices per plastic bag. By processing Thai traditional tobacco, the farmers will be exposed to nicotine in tobacco leaves and may be at risks of health effects caused by GTS. As for Thailand, it is worth noting that a diagnostic criteria for GTS has not yet been established, and this could be a potential cause of GTS in Thai traditional tobacco farmers.

By processing Thai traditional tobacco, the farmers will be exposed to nicotine in tobacco leaves and may be at risks to health effects caused by GTS. Dealing with specific symptoms of the tobacco farmer like a GTS may be one of accumulating symptoms between nicotine and pesticide poisoning that needs to discuss for clarifying of factors lead to health adverse effect. Generally, GTS is likely to define base on questionnaire and four main kinds of subjective health symptoms, headache, nausea, dizziness, vomiting. For Thai traditional tobacco processing with maintaining tobacco plants particularly with watering activities that farmers may contacting to wet tobacco leaves which almost of them are conducting in the morning or evening that contribute to exposure with nicotine and pesticide also. Depending upon the toxic potential of the compound, routes of exposure, and exposure time, the symptoms of pesticide exposure vary from headache, vomiting, skin rash, respiratory problems, and convulsions (13). Thai farmers have been identified as a high risk group for occupational poisoning because they are used to prolonged exposure to pesticide and accumulate toxic in tobacco (14). Organophosphates and carbamates inhibit acetylcholinesterase which causes accumulation of acetylcholine at nerve endings, resulting in a cholinergic or hypersecretory syndrome (15). Acetyl cholinesterase (AChE) activity in the red blood cells and butarylcholinesterase activity in plasma have been used to monitor the extent of organophosphate and carbamates exposure (16),(17),(18). However, during tobacco plantation seasons, farmers meticulously take care of tobacco plants with fertilizers until the plants are mature. During the process, insecticides are used to protect the roots of the plants. They generally spray insecticides onto young plants when the plants are infested by insects. Organophosphate and carbamates in fertilizers may cause both acute and chronic adverse health effects to the farmers. With regard to specific symptoms of the tobacco farmers that are similar to GTS, such symptoms may result from accumulation of nicotine and pesticide poisoning. Such an issue needs to be further discussed in detail to further clarify various factors that lead to adverse health effects.

As for the Thai traditional tobacco production process, taking care of tobacco plants particularly by watering means those farmers will come in contact with wet tobacco leaves. Watering activities are usually conducted either in the morning or in the evening, and such activities cause farmers to be exposed to nicotine and pesticides that are left on tobacco leaves. The purpose of this study was to assess whether there were any risks of GTS related to AChE and plasma cholinesterase PChE inhibition due to exposure to nicotine and pesticides among Thai traditional tobacco farmers. It was anticipated that the findings of the present study could be used to explain GTS etiology for farmers. Also, the study findings would shed light on the relationship between GTS and pesticide exposure in Thai traditional tobacco farmers. Moreover, Thai traditional tobacco cultivation and production involve various processes which are very unique. It is also noteworthy that dried Thai traditional tobacco production in Thailand is different from that in the western countries.

Farmers generally get into the profession either by themselves or as family labors. In addition, nicotine may be absorbed from other routes such as inhalation that is possible in dried Thai traditional tobacco production. Simply put, coming in close contact with vapor smell of nicotine from raw and dried tobacco and working for long hours on each day put farmers at health risks. Gummy, juice, and sap from tobacco leaves that break from leaves produce a pungent odor which can be directly inhaled into the body through the respiratory tract or mucous membrane in the nasal cavity. Using bare hands to handle tobacco leaves and inhaling tobacco dust can also bring about adverse health effects or GTS (19). Besides this, inappropriate use of personal protective equipment may further increase chances of nicotine absorption (20). Put another way, in dried Thai traditional tobacco production which involves manual work, farmers are exposed to nicotine in tobacco leaves and susceptible to inhalation of nicotine dust, leading to nicotine poisoning.

However, measuring the concentration of nicotine in the body comes with a very high cost and can be done only in a laboratory. Possibility of measuring nicotine in the body by means of cotinine levels is indicated in previous studies. Cotinine is a major metabolite of nicotine and results from the metabolism of nicotine. It has a relatively long half-life (ten times longer than that of nicotine) (21). The levels of cotinine has been used to distinguish between tobacco users and non-users (22), (23), (24), (25). The existence of cotinine can be detected in human saliva (26). Quandt et al. (2001) have found that levels of salivary cotinine among tobacco workers had a significant and positive relationship with working in a wet condition, smoking, and work tasks (i.e., picking and topping or removing the flower from the plant to induce plant growth and increase nicotine content) (10). Salivary cotinine levels are generally measured with the NicAlert™ Saliva strip tests (Nymox Pharmaceutical Cooperation, St.-Laurent, QC, Canada) and it can indicates the possibility to classify cotinine levels between non-users of tobacco products and users of tobacco products. Nevertheless, it is worth noting that the Thai diagnostic criteria for GTS have not been established even though GTS is possible to occur in farmers involved in dried Thai traditional tobacco production. Furthermore, the correlation between salivary cotinine levels and GTS in Thai traditional tobacco farmers has not been identified.

The purpose of this study is to investigate the prevalence of Green Tobacco Sickness (GTS) and characteristic factors related to GTS among Thai traditional tobacco farmers and to assess whether any potential GTS exist, to relate levels of inhibition acetyl cholinesterase (AChE) and plasma cholinesterase (PChE) and/or exposure to the pesticide on Thai traditional tobacco farmers. To investigate the correlation between GTS and personal protective behaviors among Thai traditional tobacco farmers. To determine the correlation between GTS from dry Thai traditional tobacco producing and salivary cotinine levels among Thai traditional tobacco farmers at Praputthabath Sub-District, Chiangklang District and Phatow Sub-District, Thawangpha District, Nan Province, Thailand. Data from this study can be helpful to expand surveillance and prevention of GTS, explain GTS etiology for farmers and improving working conditions in this area.

1.2 Research question of the study

1. What is the prevalence of GTS on Thai Traditional Tobacco Farmers in season 2012/2013?

2. What are the salivary cotinine levels and their association with GTS among Thai traditional tobacco farmers?

3. What are the pesticide exposure levels and their association correlation with GTS among Thai traditional tobacco farmers?

4. Does the relationship between the personal protective behaviors and GTS among Thai traditional tobacco Farmers?

1.3 Objectives of the study

The general objective of this study is to ascertain the health impact of Thai Traditional Tobacco cultivation and dry processing as GTS; subjective health symptoms on occupational exposure by Nicotine absorption from tobacco leaves in Nan Province, the Northern of Thailand.

The specific objectives of this study are to

1. To investigate the prevalence of GTS among Thai Traditional Tobacco farmers.

2. To measure the salivary cotinine levels by NicAlert™ Saliva strip test (NCTS) on Thai traditional tobacco farmers.

3. To measure the pesticide exposure levels by blood acetylcholinesterase (AChE), plasmacholinesterase (PChE) with the Test-Mate OP Kit (EQM Inc, Junefield, Ohio) on Thai traditional tobacco farmers.

4. To examine the relationship between the salivary cotinine levels, pesticide exposure levels, personal protective behaviors levels and GTS among Thai traditional tobacco farmers in Nan Province, Thailand.

1.4 Hypotheses

1. The Salivary cotinine levels, pesticide exposure levels and personal protective behaviors have significantly associated with the GTS among Thai traditional Tobacco Farmers.

1.5 Benefits of the study

1. To strength the evidence on health effects of tobacco farming and health of Thai Traditional Tobacco farmers.

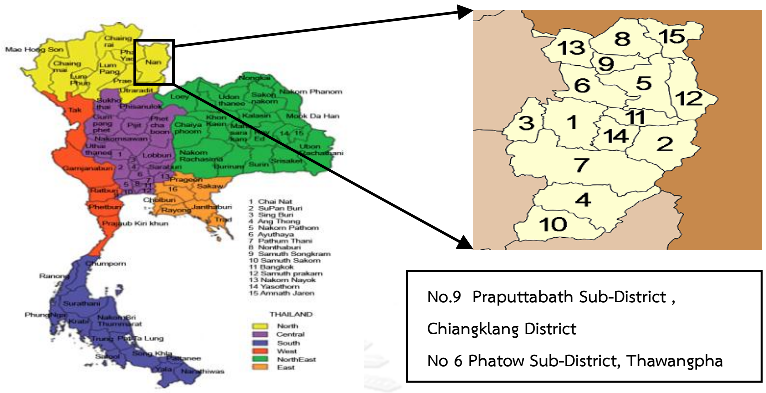
2. To Increase public awareness about the harmful effects of tobacco growing, harvesting and producing dry tobacco.

3. To recommended for protection the health of individuals working in traditional tobacco production.

4. To Prevent of health adverse, explain etiology in Thai traditional tobacco farmers.

1.6 Study area

This study conducted in Praputthabath Sub-District, Chiangklang District and Phatow Sub-District, Thawangpha District, Nan Province, Thailand.



1.7 Variables in the study

1.7.1 Independent variables

Socio-demographic

General characteristics concentrated on gender, age, education level, marital status, health problems and occupation.

Dermal exposure to Nicotine

To focused on about the contact with tobacco (Type of work , season), Skin area exposed (work with no long shirt), Surface area to volume ratio ; Body Mass index (BMI), Protective clothing (wear rain suit), Dry conditions (change out of wet clothes), Work experience (year worked in tobacco).Contact with tobacco through work, skin exposure, land preparation, seeding, planting, taking care of the tobacco leaves, watering, budding (removing axillary buds), topping(cutting a top of tobacco plant), drop herbicide for control budding, spraying insecticide, harvesting (picking), hold in arms or axilla, transporting, curing tobacco leave, drawing stem ripen tobacco leaves, rolling bundle ripen tobacco leaves, slicing ripen tobacco leaves, spread out on bamboo rack , dry in the sun, reverse bamboo rack, fold tobacco line, packaging in plastic bag, storing.

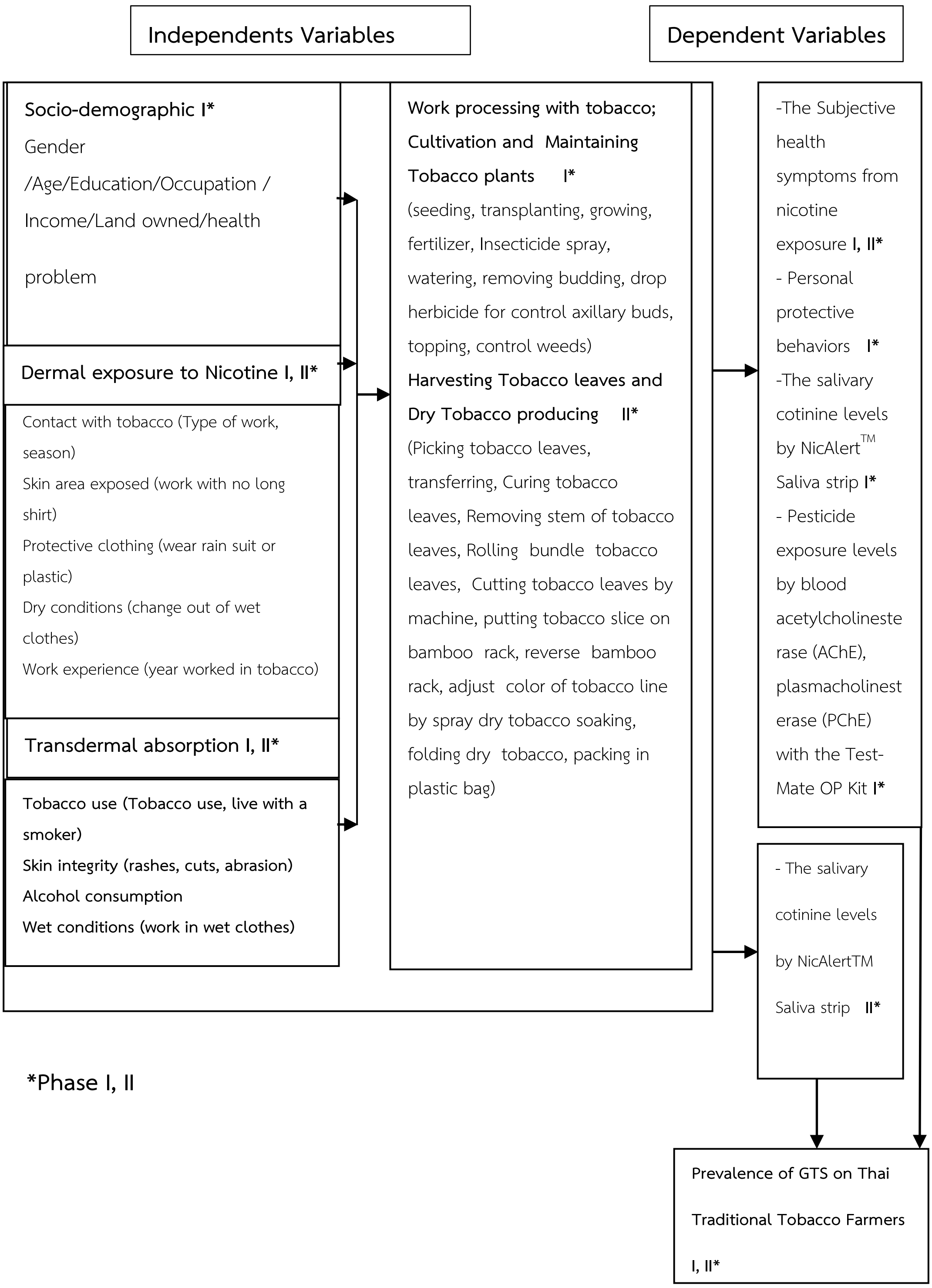
Transdermal absorption

Tobacco use (Tobacco use, live with a smoker), Skin integrity (rashes, cuts, abrasion), Alcohol consumption, Wet conditions (work in wet clothes)

1.7.2 Dependent variables

- Prevalence rate of GTS on Thai traditional tobacco farmers.
- Salivary cotinine levels by using the NicAlert™ nicotine test.
- Pesticide exposure levels by blood acetylcholinesterase (AChE), plasmacholinesterase (PChE) with the Test-Mate OP Kit.
- Subjective health symptoms from nicotine exposure and personal protective behavior among Thai traditional Tobacco Farmers.

1.8 Conceptual framework



Adapted from Bio- behavioral model of green tobacco sickness (GTS) (Arcury et al., 2001 and Quandt et al., 2000

1.9 Operational Definitions

Green Tobacco sickness (GTS) refers to symptoms after expose to tobacco leaves within 2-3 days with typical symptoms include: **Common**; headache, nausea, vomiting, dizziness, **Others**; blurred vision, weakness, runny eyes, increased salivation and increased perspiration (2), (20), (27), (28).

The subjective health symptoms from Nicotine Exposure refer to

Headache refers to a pain in the head with the pain being above the eye or ears, behind the head (occipital), or in the back of upper neck.

Nausea refers to a person with nausea has the sensation that he or she might vomit. Nausea almost always occurs before vomiting.

Dizziness refers to a sensation of temporary imbalance without spinning. Usually the duration is short: a range of few second to few minutes. The patient is afraid of falling on the floor or ground, but usually he or she succeeds to prevent falling.

Vomit refers to the reflex act of ejecting the contents of the stomach through the mouth.

Weakness refers to any of several conditions characterized by lack or loss of strength and energy.

A runny eye refers to the reflex act of eyes released teardrop after working with tobacco leaves.

Blurred vision refers to a lack of sharpness of vision resulting in the inability to see fine detail.

Increased perspiration refers to the secretion of fluid by the sweat glands. Perspiration severe at least two purpose: the removal of waste products such as urea and ammonia, and cooling of the body temperature as sweat evaporates.

Increased salivation refers to a person with excess salivation produces abnormally large amounts of saliva.

Thai traditional tobacco farmers refers to Thai agriculturist who growing, harvesting and producing dry tobacco with Thai traditional tobacco type in Nan province, Thailand. In the seasonal 2012/2013

Occupational Expose to Nicotine refers to expose by dermal absorption and transdermal absorption from Thai traditional tobacco leaves in during process and time work.

A salivary Cotinine level refers to Nicotine absorption from tobacco leaves and metabolite in saliva on the Thai traditional tobacco Farmers by using the NicAlert™ Saliva strip test.

Pesticide exposure levels refers to blood acetylcholinesterase (AChE) levels and plasmacholinesterase (PChE) levels by the Test-Mate OP Kit

Smokers refer to persons who consume at least one cigarette per day every day.

Passive smokers refers to persons who indirectly affected by contaminated air with environmental tobacco smoke (ETS).

CHAPTER II LITERATURE REVIEW

2.1 Green Tobacco Sickness (GTS) and the subjective health symptoms from Nicotine

2.1.1 Symptoms

During GTS onset, early symptoms often include headache and nausea followed by vomiting, weakness, pallor, dizziness, headaches, increased perspiration, chills, abdominal pain, diarrhoea, and increased salivation. (5), (9), (22), (28), (29), (30). These effects can be rather extreme, and may also include severe prostration (1), (26) . Shortness of breath, and occasional fluctuations in blood pressure or heart rate (5), (7). Among those susceptible, the average length of the illness, with treatment, is between one and three days (median = 2.4 days) (30), (31). Some of the symptoms of GTS are similar to those of organophosphate poisoning and heat exhaustion. However, many of the symptoms of organophosphate poisoning (including increased lacrimation, pulmonary edema, and meiosis) have not been associated with GTS (9). The possibility that GTS symptoms are due to pesticide poisoning is lessened because the last application of pesticides normally occurs several weeks before harvest (9), (29), (30), (31), (32), (33) Because GTS is known to occur among workers on farms that do not use pesticides. Furthermore, cases of GTS were documented before widespread pesticide use (32). Symptoms of heat exhaustion have been ruled out in many cases of GTS. Although tobacco is typically harvested during hot weather, GTS symptoms have also appeared during cool conditions when harvesters reported feeling chilled rather than overheated (30). Also, most of those stricken with GTS became ill after they had gone home for the day (median onset = 10 hours) (33).

2.1.2 Etiology; Exposure to nicotine Burley and flue-cured tobacco are the two main types of tobacco grown in the United States, accounting for 94% of all tobacco grown. Burley is grown primarily in Kentucky and Tennessee, whereas flue-cured tobacco is grown largely in North Carolina, South Carolina, Virginia, and Georgia (28, 34). The amount of nicotine present in a tobacco leaf depends on a number of factors including genetics, soil, fertilization practices, weather, and cultivation and harvesting techniques(9); (35). Nicotine levels in dark varieties of tobacco such as dark fire-cured, dark air-cured—are generally higher than burley (36). GTS occurs primarily among tobacco workers who hand-harvest (“crop”) tobacco leaves in the field and handles the leaves as they are placed in barns for curing. The process of cropping flue-cured tobacco usually consists of pulling and twisting loose green leaves from the plant and collecting them in large bundles that are held either in the hand or underneath the arm and against the body. For burley tobacco, the entire stalk is removed and the tobacco is typically held in the hand or on the forearm. Hand harvesting can lead to skin abrasions, further increasing risk of contracting GTS. Larger farm operators are increasingly using mechanical harvesting equipment, thus reducing dermal exposure to tobacco leaves. Cropping typically occurs in the summer and autumn months. Workers begin in the early morning, when the tender green tobacco is wet with dew. GTS occurs primarily when people handle wet tobacco (2), (26), (30), (31). The geographical clustering of GTS cases is influenced by rainfall, temperature, and humidity (2). In the process of cropping tobacco, leaves and stalks are often cracked, emitting a gummy substance that coats workers’ hands, skin, and clothing (32). Although tobacco is handled during many stages of production, GTS occurs primarily among workers who handle green leaves and stalks in the field or during the process of transferring green tobacco to the curing barn (30).

2.1.3 Absorption of nicotine; GTS is a threat to those who harvest tobacco because nicotine, being soluble in water, can be drawn out of tobacco by rain, dew, or perspiration, and subsequently absorbed through the skin (5) ; (22). As much as 9 mg nicotine may be contained in 100 ml of dew (5). Although there is no accurate measurement of the amount of nicotine-laden dew to which tobacco harvesters are exposed, Gehlbach and colleagues suggested that 600 ml would be a conservative estimate (5). The percentage of dew-laden nicotine absorbed transdermal, however, is not known. Despite this, many studies have documented the increase of cotinine (a nicotine metabolite) in the urine of tobacco workers, after controlling for those who reported regular tobacco usage (2), (5), (22). Absorption was found to be greatest among croppers who had the most contact with the wet leaves and least among stringers (those who tie burley tobacco leaves on poles for curing) and tractor drivers. Once the dew-laden tobacco is contacted, Croppers can absorb a great deal of nicotine in a relatively short period of time. It has been reported that nausea and faintness can occur within 15 minutes of skin contact (37). Although the US Centers for Disease Control and Prevention (CDC) reported that the median time from exposure to onset of GTS was 10hours (ranging from three to 17 hours) (1), (2).

2.1.4 Effects of nicotine; once nicotine is absorbed, it is distributed throughout the body, including into the brain. The nausea and vomiting characteristic of GTS is mediated by the direct action of nicotine on the emetic chemoreceptor trigger zone in the medulla oblongata leading to reflex vomiting (21). Nicotine also excites sensory nerves from the gut and parasympathetic nerves in the gastro intestinal tract, which lead to an overall increase in gastrointestinal secretion and motility. The pharmacological effects of nicotine on nicotinic receptors in the central nervous system and at post-synaptic autonomic ganglia have been well elaborated (38) and help to explain the toxic effects of nicotine. However, symptoms associated with severe nicotine poisoning, such as convulsions, dyspnea, and vascular collapse, are not typically seen in GTS cases (30). Symptoms that are ascribed to nicotine intoxication in novice smokers mimic green tobacco sickness for example, nausea, vomiting, increased heart rate, chills.

2.1.5 Epidemiology; A few studies have estimated the incidence of GTS. Using United States and Kentucky Department of Agriculture data, the incidence of GTS was estimated to be 10/1000 workers (or 1%) (26). In 1973, a study in North Carolina estimated a 9% prevalence of GTS 5400 of 60 000 workers (30). These estimates are not comparable because case numbers were based on self-reported data in North Carolina and on hospital-treated cases in Kentucky. Thus, a true estimate of the prevalence of GTS is difficult to derive because reporting methods are not standardized and many cases likely go unreported. Younger workers are more likely than older workers to develop GTS. (26), (30), (39). In one study, 58% of those suffering GTS were under age 29 and 32% were between 14 and 19 years of age (39). Likewise, it was found that younger people (under age 30) were 3.1 times more likely to develop GTS than older people (26). Differences by gender have also been found

(23), (30), (39). Nearly all of those affected by GTS are male (30), (39). Although women do not have any special genetic protection. Sex differences are probably due to the fact that women are largely under-represented among tobacco croppers (2) . Familial clustering of GTS has also been found (1), (30), (40). This may be less a function of genetic predisposition and more a function of the fact that in regions where there is little mechanization, such as on small family farms, families or groups of individuals must manually harvest the crop under similar conditions, which in turn may lead to similar exposure patterns. Along these lines, GTS is known to recur among those susceptible to the illness (5), (9), (23), (32), (40). Gehlbach and colleagues reported that as many as 12 recurrences over eight weeks have been reported by some workers (5). There is a discrepancy in the literature between the susceptibility of tobacco users and non-users. In some studies, GTS was found to be less likely to occur among those who were current tobacco users, perhaps resulting from an increased tolerance to the effects of nicotine (5), (9), (26), (30). This seemingly acquired tolerance, however, may not be completely protective if the cropper's typical nicotine exposure is significantly exceeded (2), (25), (26). In contrast, a few studies have suggested that active smoking offers no protection against GTS (23), (41). One study found that tobacco users in India actually had a higher prevalence of "green symptoms" than non-tobacco users (41).

2.1.6 Diagnosis and Treatment ; Because GTS is self-limiting and of short duration (30), treatment is not always necessary. Despite the relatively short duration of GTS, the illness can be debilitating during its onset and progression. Clinical diagnosis of GTS is based upon both the presence of symptoms described above and a history of harvesting tobacco. The diagnosis of GTS may be made by testing the blood or urine for nicotine (half-life = 3–4 hours) or cotinine (a nicotine metabolite (half-life = 36 hours) that can also be detected in saliva) (26). Although the level of cotinine has been used to distinguish between tobacco users and non-users, (22), (23), (25). The level cannot be used to distinguish between heavy tobacco users and persons with GTS, because nicotine/cotinine concentrations that represent toxic levels have not been established (26). Little has been written about the treatment modalities available to those seeking relief from GTS. Although it can take as much as 10 hours before GTS symptoms occur, the most common suggestion once symptoms occur is to avoid increased contact with green tobacco. This can be accomplished by ceasing work, changing clothes, and showering. In addition, exposed workers are encouraged to increase fluid intake, ingest Dimenhydrinate (Dramamine), and rest (9), (39). The therapeutic effects of H1 blockers such as dimenhydrinate, however, are not mediated through an antagonistic action on the nicotinic cholinergic receptors. When symptoms are serious, physicians can administer intravenous hydration, anti-emetics, and H1 blockers (Dimenhydrinate) (1), (39), (40).

2.1.7 Costs ; Because nearly a quarter of those stricken with GTS who sought medical treatment required hospitalization (2), (39). Significant hospital expenditures are associated with the condition. GTS induced hospital expenses are estimated to average US$250 for outpatient treatment, $566 for hospital admission, and $2041 for intensive care treatment (2). These figures do not include costs associated with lost income and productivity incurred by someone's inability to work. Because nearly half of Kentucky tobacco harvesters are employed off-farm (in work unrelated to farming) (39). Financial loss from missed work due to GTS is compounded further.

2.1.8 Risk Reduction; Despite the awareness of GTS among some clinicians and tobacco workers, very little widespread action has been taken to reduce the risks associated with harvesting tobacco. If a worker becomes ill while working with tobacco and requires medical attention, the physician should be informed of the exposure to nicotine to aid in diagnosis, as it is common to misdiagnose GTS as pesticide poisoning or heat exhaustion. The use of protective, water resistant clothing and chemical-resistant gloves would reduce the amount of nicotine absorbed by workers in contact with green tobacco (1), (2), (22), (23), (25), (26), (40), (41). Current occupational health regulations do not require this level of protection. It has been suggested that croppers should avoid harvesting in the rain or should begin harvesting after the dew evaporates (30). Plastic aprons and rain suits, in addition to boots and socks, (23) have been used to reduce exposure to nicotine (25). These actions must be weighed against the increased risk of heat stress caused by wearing impermeable clothing in hot weather (2), (23) . Additionally, dimenhydrinate is useful in treating GTS once onset has occurred and as a prophylactic measure before harvesting tobacco (42). The CDC advises tobacco farm operators to inform their employees of the hazards associated with harvesting wet tobacco and the importance of safe work practices in preventing GTS (2), (43) but it is unclear how many operators take such action. Mechanization of tobacco harvesting will reduce skin contact with wet tobacco leaves and represents a potential method for prevention, although equipment for mechanical harvesting is not accessible to smaller farm operations.

2.1.9 Public Health Response; Very little regulatory effort has been undertaken to address the potential hazards of GTS. Currently, there is no legal requirement that workers be informed about the hazards of nicotine exposure (44). In Kentucky in 1992, for example, an Occupational Health Nurses in Agricultural Communities study of GTS was undertaken to educate tobacco workers and healthcare providers about the dangers inherent in tobacco harvesting (2). The educational effort included targeted informational mailings and news stories in the local media, coinciding with the tobacco harvest. Following a public awareness campaign, the 1993 incidence of hospital-treated GTS increased from the previous year, probably due to the heightened awareness about GTS on the part of tobacco workers and healthcare providers. This study suggests that in the absence of an educational intervention, the magnitude of GTS may not be fully recognized.

2.2 Bio-behavioral model of green tobacco sickness causation

Quandt and colleague (10), (45), (46) present a bio-behavioral model of green tobacco sickness (Figure) based on the existing green tobacco sickness research and on the physiology of percutaneous absorption of nicotine and other chemicals (47-50). They argue that green tobacco sickness results from the rate of transdermal absorption of nicotine, determined by the amount of dermal exposure to tobacco plants as well as several other factors. Dermal exposure to nicotine is increased by greater skin exposure and work activities that increase contact with the plants. Wearing protective clothing (for example, a plastic rain suit) decreases exposure, as does learned avoidance gained through work experience with tobacco (23). The relation of dermal exposure to transdermal absorption was mediated by several factors. Compromised skin integrity (for example, cuts, rashes) (23) may increase absorption, as may factors that increase vasodilatation, particularly consumption of alcoholic beverages and work in hot and humid weather. Working in wet tobacco also increases dermal absorption because nicotine is water soluble (47), (51). Use of tobacco products (smoking or smokeless) seems to decrease absorption (5), (25), (26).

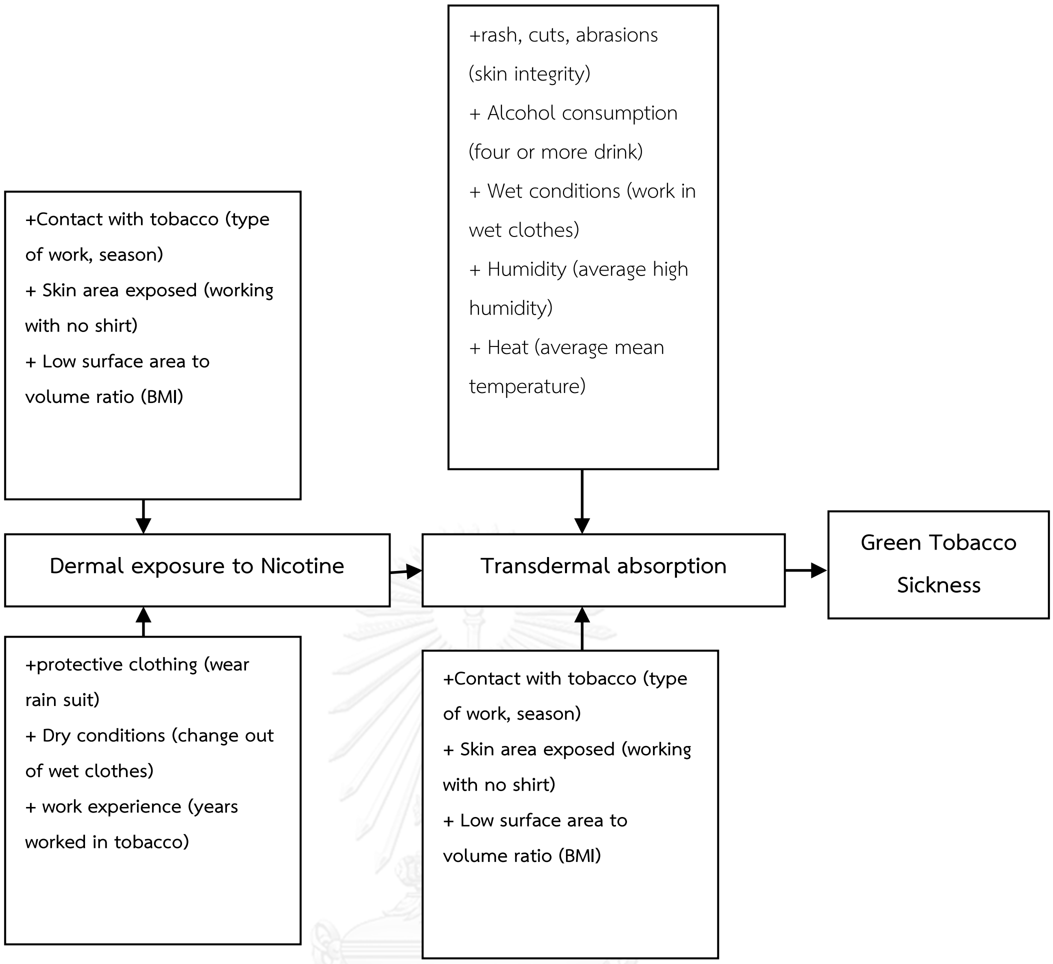


Figure 1 Bio-behavioral model of green tobacco sickness causation (based on Quandt *et al*., 2000)

2.3 Dermal and Transdermal Exposure in tobacco farmers

The level of plasma nicotine results from the rate of transdermal absorption of nicotine, determined by the amount of dermal exposure to tobacco plants and several other factors. Dermal exposure to nicotine was increased by greater skin exposure and by work activities that increase contact with the plants. Wearing protective clothing decreases exposure, as does learned avoidance gained through work experience with tobacco (23). Several factors mediate the relationship between dermal exposure and transdermal absorption. Compromised skin integrity may increase absorption (23), as may factors that increase vasodilation, particularly consumption of alcoholic beverages and work in hot and humid weather. Working in wet tobacco also increases dermal absorption because nicotine is water soluble (47), (51). Use of tobacco products (smoking or smokeless) appears to decrease absorption (5), (25), (26), (30), (49).

In earlier analyses, Arcury and colleagues(10), (27) determined the prevalence and incidence of GTS, and they tested the parts of their bio-behavioral model that link dermal exposure and dermal absorption variables to GTS symptoms and to salivary cotinine levels. The prevalence of the GTS syndrome among Latino farm workers in North Carolina is 24.2%, and the incidence density was 1.88 GTS events for every 100 days of exposure (27). The dermal exposure risk factors found to be significantly related to increased GTS incidence include work task (picking tobacco vs. other tasks), period of the growing season (middle and late vs. early), working in wet clothes, and limited tobacco work experience. The dermal absorption variable ‘‘temperature’’ increased GTS incidence (higher vs. lower temperature). The dermal absorption variable ‘‘smoking tobacco’’ had a significant inverse relationship to GTS incidence (45). Although smoking tobacco was considered a dermal absorption variable due to its vasoconstrictive action, the model assumes tobacco use was related to metabolic adaptation or tolerance that will reduce the effect of dermally absorbed nicotine on the incidence of

GTS. That the level of salivary cotinine among these workers had a significant positive relationship to greater age, later season work, wet working conditions, smoking, and work task (picking vs. topping [removing the flower from the plant to induce plant growth and increase nicotine content]). In a multivariate analysis of cotinine levels, they found that these factors accounted for 69% of the variance in cotinine on the natural log (ln) scale (10).

2.4 Tobacco Cultivation and Dry Tobacco Producing

2.4.1 Thai Traditional Tobacco Cultivation and Dry Tobacco Producing in Nan Province

The traditional cultivation of local type of tobacco was a result of the accumulated local wisdom passed on from generation to generation. In the rural community, the traditional tobacco type has been exclusively used for smoking as well as everyday social activities and welcoming important guests. The appropriate land used for tobacco plantation was either wetland or lowland with sufficient water irrigation. Process of Thai traditional tobacco cultivation and maintaining are specific and refinement. Process of all starting to seed cultivates and transplant to field, maintained by fertilizer, insecticide, removing axillary buds, topping, get rid of weeds.

Table 3 Processing of Thai Traditional Tobacco Cultivation and Maintaining

No	Process	Time	Body Expose	Working conditions
1	Seeding and take care of tobacco plants	3 Months	Hands /feet	Wet
2	Growing tobacco plants	1 Time	Hands	Wet
3	Fertilizer feeding	After growth 20 days	Hands	Hot /dry
4	Control of soil pathogens and/or grasses /pesticide are seldom applied	seldom applied ~ 3 times	Hands/skin/feet	Wet/dry
5	Topping (cutting top of tobacco plants) increase leaves weight and nicotine content	1 Time	Hands /body/feet/	Wet/dry
6	Removing axillary buds	Every week	Hands /body/feet/	Wet /dry
7	Picking priming tobacco leaves	1 times	Hands /body/feet/	Dry /Hot
8	Herbicide drop for control top and axillary buds of tobacco plants	1 time	seldom applied	wet
9	Watering tobacco plants	Every week	Hands /body/feet/	Wet

Harvesting of Thai traditional tobacco at 100-120 days, tobacco was a high yielding profitable crop in the short period of time. After that curing between 5 and7 days until ripen, remove a stems of ripen tobacco leaves by draw and rolling tobacco leave prepare to slice with cutting machine , bring tobacco slices put on bamboo racks and dry in the sun 1-3 days, in every day and night the farmers must reverse the bamboo racks for control the color of tobacco line and adjust it by spray a dry tobacco soaking, in the nigh a dew will make tobacco slice soft and easy to fold then packaging in plastic bag per 10 kilograms waiting for selling to the merchant.

Table 4 Processing of Harvesting and Dry Thai Traditional Tobacco Producing

No	process	Time	Body Expose	Working conditions
1	Harvesting Tobacco Leaves (picking tobacco leaves) (sap and gummy)	All day almost start at 9 A.M. to en d of the day	Hands /forearms/things/ face /axilla/back/feet	Dry
2	Carry Tobacco Leaves (gummy)	1 Day	Hands/	Dry
3	Sort out and Fold Tobacco Leaves (gummy)	1 Day	Hands/skin	Dry

Table 5 Processing of Harvesting and Dry Thai Traditional Tobacco Producing (Cont.)

No	process	Time	Body Expose	Working conditions
5	Curing Tobacco Leaves (gummy)	5-7 Day	Hands	Dry
6	Pull a stem of Tobacco Leaves and rolling a bundle of tobacco leaves (gummy/pungent odor)	1 Day	Hands/skin /inhalation	Dry /humid
7	Cutting Tobacco Leaves by cutting machine (gummy/pungent odor)	1 Day	Hands /inhalation	Dry/humid
8	Putting Tobacco slices on bamboo rack (gummy/pungent odor)	1 Day	Hands /inhalation	Humid
9	Put tobacco rack dry in the sun/adjust and decorate the color (pungent odor)	1 Day	Hands	Dry
10	Reverse tobacco rack (dust/pungent odor)	Day/Night	Hands /inhalation	Humid
11	Fold tobacco line in bamboo rack as a piece (dust/pungent odor)	In the night	Hands /inhalation	Humid
12	Pack in plastic bag for sell (dust/pungent odor)	1 Day	Hands /inhalation	Dry

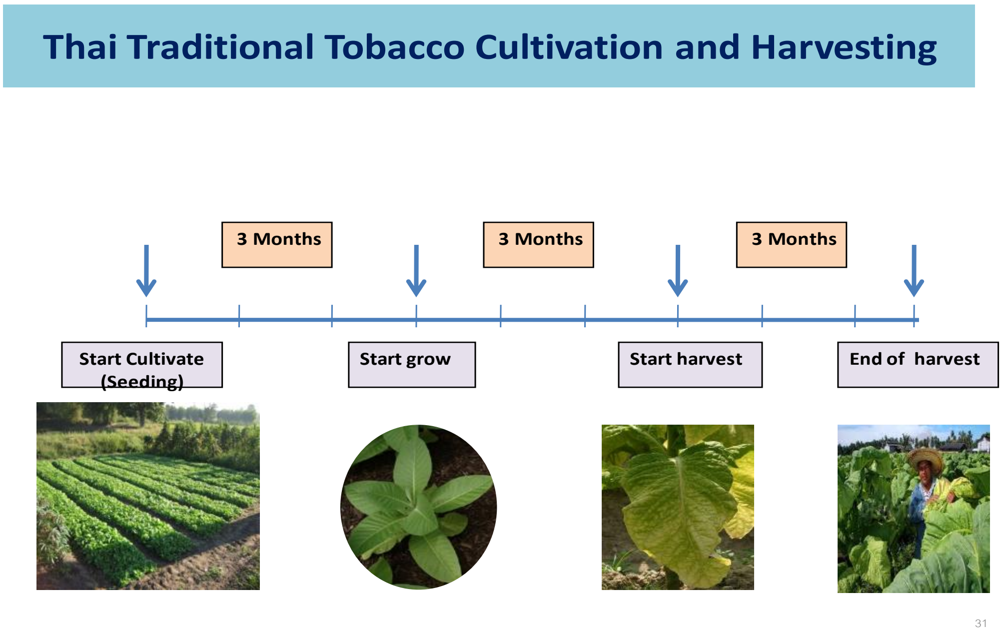


Figure 2: Time frame of Thai Traditional Tobacco Cultivation and Harvesting in Nan Province

2.5 Nicotine Chemical substance

2.5.1 Nicotine Chemical substance

Brand names, Trade names

Nicabate, Nicobrevin, Nicotinell TTS, Nicorette, Nicoret, Cigarette tobacco, Black leaf, Nicocide, Nico-fume. Transdermal patches deliver 5 to 30 mg nicotine over 24 hours; used patch has significant nicotine content. Cigarette tobacco varies in its nicotine content but common blends contain 15 to 25 mg per cigarette with a current trend towards lower levels. Nicotine insecticides: 40% solution of the sulfate. Chewing gum - nicotine polacrilex: 2 and 4 mg nicotine bound to an ion exchange resin in a sugar-free flavored chewing gum base (43).

Summary

Main risks and target organs

Nicotine is one of the most toxic of all poisons and has a rapid onset of action. Apart from local caustic actions, the target organs are the peripheral and central nervous systems. Nicotine is also a powerfully addictive drug.

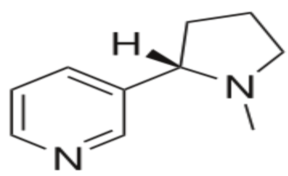
Summary of clinical effects; Burning sensation in the mouth and throat, salivation, nausea, abdominal pain, vomiting and diarrhoea. Gastrointestinal reactions are less severe but can occur even after cutaneous and respiratory exposure. Systemic effects include: agitation, headache, sweating, dizziness, auditory and visual disturbances, confusion, weakness and lack of coordination. A transient increase in blood pressure, followed by hypertension, bradycardia, paroxysmal atrial fibrillation, or cardiac standstill may be observed. In severe poisoning, tremor, convulsions and coma occur. Faintness, prostration, cyanosis and dyspnoea progress to collapse. Death may occur from paralysis of respiratory muscles and/or central respiratory failure.

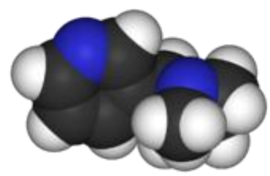
First-aid measures and management principles; There were no known antidotes. Immediate establishment of an airway, monitoring of breathing patterns, and maintenance of circulation are essential in cases of serious overdose. Preparations for possible seizures or rapid progression to coma and artificial ventilation procedures should be kept ready, oxygen may be required. If vomiting has not occurred following nicotine ingestion, remove stomach contents by gastric lavage. Induction of emesis is less preferable to lavage since convulsions or coma may intervene. Single or multiple doses of activated charcoal may be used. Children who ingest more than one cigarette should receive activated charcoal and medical observation for at least several hours. If nicotine is spilled on the skin, immediately wash thoroughly with running water (avoid warm water).Seizure activity and agitation can be controlled with diazepam or barbiturates. Cholinergic symptoms may be ameliorated with atropine (43).

Physicochemical properties

Origin of the substance; Nicotine is a natural alkaloid obtained from the dried leaves and stems of the Nicotiana tabacum and Nicotiana rustica, where it occurs in concentrations of 0.5-8%. Cigarette tobacco varies in its nicotine content, but common blends contain 15-25 mg per cigarette, with a current trend towards lower (52)

Chemical structure : $C_{10}H_{14}N_2$





Physical properties

Molecular weight: 162.26

Nicotine is a liquid alkaloid. It is water soluble and has a pKa of 8.5. It is a bitter-tasting liquid which is strongly alkaline in reaction and forms salts with acids.

Other characteristics; Store at room temperature, below 86 F (30°C). Protect from light and air (52).

Use /circumstances of poisoning; Nicotine is most frequently encountered in tobacco products for smoking, chewing, sniffing and tobacco "without smoking". As an insecticide (now rare), and as an adjunct to smoking cessation programs (gums, patches). It is a substance of abuse.

Occupationally exposed populations; People who are involved in the processing and extracting tobacco (green tobacco sickness), as well as mixing, storing and applying certain insecticides (52).

Routes of entry

Oral; Poisoning occurs in children who ingest cigarettes or cigars or 2nicotine gum. In adults chewing tobacco or nicotine gum, and people who ingest liquid nicotine in the form of insecticide preparations.

Inhalation; Inhalation is the most frequent route of entry because of worldwide tobacco smoking.

Dermal; dermal exposure to nicotine can lead to intoxication. Such exposure has been reported after spilling or applying nicotine containing insecticides on the skin or clothes (52), (53), and as a consequence of occupational contact with tobacco leaves (green tobacco sickness) (30), (32).

Others ;Tobacco has been used in enemas and poultices (54).

Kinetics

Absorption by route of exposure; Nicotine is a water and lipid soluble drug which, in the free base form, is readily absorbed via respiratory tissues, skin, and the gastrointestinal tract. Nicotine may pass through skin or mucous membranes when in alkaline solution (in which nicotine is largely unionized). When tobacco smoke reaches the small airways and alveoli of the lung, the nicotine is rapidly absorbed. The rapid absorption of nicotine from cigarette smoke through the lungs occurs because of the huge surface area of the alveoli and small airways, and because of dissolution of nicotine at physiological pH (approximately 7.4) which facilitates transfer across cell membranes. Chewing tobacco, snuff, and nicotine polacrilex gum are of alkaline pH as a result of the selection of appropriate tobacco and/or buffering with additives by the manufacturers. The alkaline pH facilitates absorption of nicotine through mucous membranes (55).

Distribution by route of exposure; after absorption, nicotine enters the blood where, at pH 7.4, it is about 70% ionized. Binding to plasma proteins is less than 5%. Studies showed that, after intravenous administration, the distribution of C^{14}-labeled nicotine is immediate, reaching the brain of mice within 1 min. after injection. Similar findings based on positron emission tomography of the brain, were seen after injection of ^{11}C-nicotine in monkeys. (43). Nicotine inhaled in tobacco smoke enters the blood almost as rapidly as after rapid I.V. injections. Because of delivery into the lung, peak nicotine levels may be higher and lag time between smoking and entry into the brain shorter than after IV injection (56).

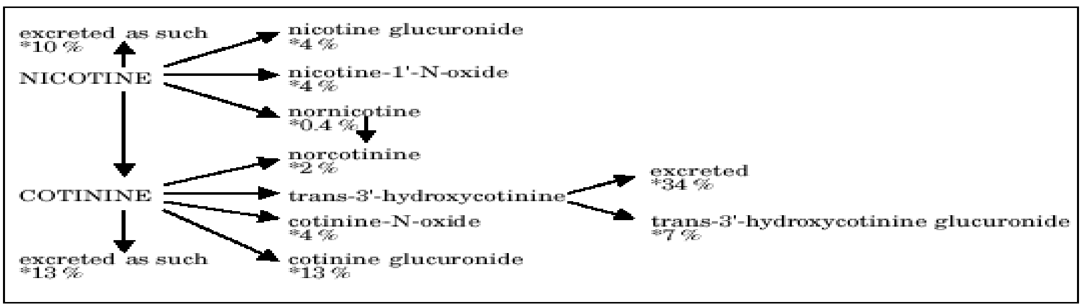
After smoking, the action of nicotine on the brain is expected to occur quickly. Rapid onset of effects after a puff is believed to provide optimal reinforcement for the development of drug dependence. The effect of nicotine declines as it is distributed to other tissues. The distribution half-life, which describes the movement of nicotine from the blood and other rapidly perfused tissues, such as the brain, to other body tissues, is about 9 min (57).

Distribution kinetics, rather than elimination kinetics (half-life about 2 hrs.) determine the time course of the CNS actions of nicotine after smoking a single cigarette. The apparent volume of distribution in animals is approximately 1.0 L/kg whereas in one clinical study it was, 2.0 L/kg in smokers and 3.0 L/kg in nonsmokers (58).

Biological half-life by route of exposure; The elimination half-life of nicotine averages 2 hours (57), (59). The half-life of a drug is useful in predicting the rate of accumulation of that drug in the body with repetitive dosing and the time course of decline after cessation of dosing. Consistent with a half-life of 2 hours, accumulation of nicotine over 6 to 8 hours during regular smoking and persistence of significant levels of nicotine in the blood for 6 to 8 hours after cessation of smoking, i.e. overnight, has been observed (60). Thus, cigarette smoking represents a situation where the smoker is exposed to significant concentrations and possibly pharmacological effects of nicotine for 24 hours a day. Apparent acute tolerance to nicotine, determined on the basis of observations of the relationship between venous blood levels and effects, may be due to distribution disequilibrium between venous and arterial blood; venous blood levels substantially underestimate concentrations of nicotine in arterial blood and at potential sites of action. True tolerance does, however, develop rapidly, with a half-life of development and regression of about 35 minutes. The kinetics of tolerance may be another determinant of cigarette smoking particularly when the smoker smokes his next cigarette.

Elimination by route of exposure; Nicotine and its metabolites (cotinine and nicotine 1-N-oxide) are excreted in the urine. At a pH of 5.5 or less, 23% is excreted unchanged. At a pH of 8, only 2% is excreted in the urine. The effect of urinary pH on total clearance is due entirely to changes in renal clearance (58). Nicotine is secreted into saliva. Passage of saliva containing nicotine into the stomach, combined with the trapping of nicotine in the acidic gastric fluid and reabsorption from the small bowel, provides a potential route for enteric nicotine recirculation. This recirculation may account for some of the oscillations in the terminal decline phase of nicotine blood levels after I.V. nicotine infusion or cessation of smoking. Nicotine freely crosses the placenta and has been found in amniotic fluid and the umbilical cord blood of neonates. Nicotine is found in breast milk and the breast fluid of non-lactating women and in cervical mucus secretions (43).

2.5.2 Nicotine Metabolism; Nicotine is a tertiary amine which is composed of a pyridine and a pyrolidine ring. Nicotine undergoes a large first pass effect during which the liver metabolizes 80% to 90%; to a smaller extent, the lung also is able to metabolize nicotine. The major metabolite of nicotine is cotinine; nicotine-1'-N- oxide is a minor metabolite. Cotinine is also extensively metabolized and trans-3'-hydroxycotinine is it's a major metabolite. The most abundant metabolite in the mice is trans- 3'-hydroxy-cotinine, accounting for almost 40%, whereas cotinine itself accounts for only about 15% of the dose of nicotine. Cotinine levels in various biological fluids are widely used to estimate intake of nicotine in tobacco users. The usefulness of cotinine as a quantitative marker of nicotine intake is limited by individual variability in percentage conversion of nicotine to cotinine and in rate of elimination of cotinine itself. Since it accounts for a much greater percentage of nicotine, trans-3'-hydroxycotinine measurement, either alone or in combination with measurement of other metabolites, may be a superior quantitative marker of nicotine intake.



2.5.3 Nicotine Toxicity

2.5.3.1 Acute toxicity; In experimental animals, the dose of nicotine which is lethal to 50 % of the animals (LD50) varies widely, depending on the route of administration and the species used. The intravenous (i.v.) LD 50 dose of nicotine in mice is 7.1 mg kg^{-1} body weight (61). By direct i.v. administration the LD 50 to rats was determined to 1 mg kg^{-1} (62). The intra peritoneal (i.p.) LD 50 values for nicotine in mice and rats have been found to be 5.9 mg kg^{-1} and 14.6 mg kg^{-1}, respectively (61). The oral LD 50 dose for nicotine in rats is 50 mg kg^{-1} to 60 mg kg^{-1} (63). The wide variation in sensitivity to the toxic effects of nicotine in rodents appears to be genetically determined (64). Dermal acute toxicity (LD50) in rabbits is 140 mg kg^{-1} (64). In interpreting animal toxicity data it is important to recognise that the route of administration is an important determinant of toxicity. Rapid i.v. injections result in the highest blood and brain concentrations and produce toxicity at the lowest doses. In contrast, oral or i.p. administration requires higher doses to produce toxicity. This is due in part to pre-systemic (“first pass”) metabolism of nicotine whereby, after absorption into the portal venous circulation, nicotine is metabolised by the liver before it reaches the systemic venous circulation. Probable oral lethal dose in humans is less than 5 mg kg^{-1} or a taste (less than 7 drops) for a 70 kg person (US-EPA. 1987). It may be assumed that ingestion of 40 mg to 60 mg of nicotine is lethal to humans (US-EPA. 1987).No inhalation toxicity data are available on which to base an immediately dangerous to life or health concentration (IDLH) for nicotine. Therefore, the revised IDLH for nicotine is 5 mg m^{-3} based on acute oral toxicity data in humans and animals (56). A number of poisonings and deaths from ingestion of nicotine, primarily involving nicotine-containing pesticides, have been reported in humans (65).

Nicotine poisoning produces nausea, vomiting, abdominal pain, diarrhoea, headaches, sweating, and pallor. More severe poisoning results in dizziness, weakness, and confusion, progressing to convulsions, hypotension, and coma. Death is usually due to paralysis of respiratory muscles and/or central respiratory failure. Dermal exposure to nicotine can also lead to poisoning. Such exposures have been reported after spilling or applying nicotine-containing insecticides on the skin or clothes and as a consequence of occupational contact with tobacco leaves (47), (65). Acute intoxication may occur in children following ingestion of tobacco materials. Four children, each of whom ingested two cigarettes, developed salivation, vomiting, diarrhoea, tachypnoea, tachycardia, and hypertension within 30 min, followed by depressed respiration and cardiac arrhythmia within 40 min and convulsions within 60 min (66). All recovered and suffered no complication. Although ingestions of tobacco are common, deaths due to ingestion of tobacco are extremely rare, due to early vomiting and first pass metabolism of the nicotine that is absorbed.

2.5.3.2 Long-term toxicity

As attested to in the U.S. Surgeon General's reports since 1964, smoking causes coronary and peripheral vascular disease, cancer, chronic obstructive lung disease, peptic ulcer disease, and reproductive disturbances, including prematurity (67). Nicotine may contribute to tobacco-related disease, but direct causation has not been determined because nicotine is taken up simultaneously with a multitude of other potentially harmful substances that occur in tobacco smoke and smokeless tobacco. However, particularly now that nicotine may be prescribed in the form of gum or other delivery systems, the potential health consequences of chronic nicotine exposure deserve careful consideration.

2.6 Salivary Cotinine levels by Oral fluid assay system for nicotine use and Others Biomarker.

Determining the concentration of nicotine and cotinine in biological fluids is widely practiced in both epidemiological and clinical smoking studies (68) . Both nicotine and cotinine concentrations are used to estimate tobacco consumption, to determine exposure to environmental smoke and to validate abstinence in smoking cessation programmes (68), (69). Nicotine, when smoked in cigarettes is absorbed across buccal and nasal membranes. The drug has a fast onset of action with a half-life of 2 hand can be detected in blood, saliva and urine (68). As nicotine is a weak base (pKa of 8.0), it is present mainly in the non-ionised form in alkaline pH, and hence more easily absorbed with increased pH levels (70). Thus, changes in salivary pH will affect the amount of nicotine that is absorbed across the buccal mucosa (71). Cotinine, the major metabolite of nicotine, is widely used for estimating exposure to nicotine. This pharmacologically inactive compound has a half-life of 20 h (15 - 40 h), is slowly cleared from the body and is specific to tobacco (55), (68). Cotinine has been reported to have a pKa<5.0, and can also be detected in urine, blood and saliva (72), (73). Urinary levels of cotinine have been shown to be quite variable, due to the difference in nicotine metabolism among individuals (74). Blood provides quantitative results that can be more accurately related to dosing. However, collection of blood samples is more invasive. In many nicotine treatment trials, saliva collection is favoured over blood and urinary measures as it is easy to obtain and non-invasive (68). Saliva samples are useful for determining compliance with medication (especially in prediatric patients), for analysing the concentration of free drugs and in situations where repeated sampling is necessary. Salivary nicotine and cotinine concentration is reported to be dependent upon a number of factors. One of the factors where variability reportedly arises in salivary nicotine and cotinine concentrations is the difference in sample collection methods (21), (69). There have been a number of techniques used to collect saliva. Saliva can be collected under unstimulated (resting) or stimulated conditions. Among the reported disadvantages of collecting unstimulated saliva was insufficient volume. Most studies have employed sampling devices that aim to stimulate the production of saliva. Among the stimulated techniques, the method of stimulation has varied between using wax, sugar, lemon juice or other acidic drinks (21), (75-77). The use of stimulated saliva has an advantage over unstimulated saliva as a larger volume sample could be obtained in a short period of time. The importance of standardising saliva collection has been highlighted for research and clinical practice (21), (69), (78). Previous study reported lowered salivary cotinine concentration when the saliva collection was stimulated with wax or sugar compared to when saliva was collected without stimulation (69). No difference in salivary cotinine concentration was observed with consecutive unstimulated saliva sampling within the same subject. However, other earlier studies were less clear cut and found no difference in salivary cotinine levels whether the sample was collected stimulated or without stimulation (21). This study aimed to determine the influence of stimulated saliva collection (compared to unstimulated collection) on salivary nicotine and cotinine concentrations.

2.7 Pesticide exposure levels by blood acetyl cholinesterase (AChE), plasma cholinesterase (PChE) with the Test-Mate OP Kit

Dealing with specific symptoms of the tobacco farmer like a GTS may be one of accumulating symptoms between nicotine and pesticide poisoning that needs to discuss for clarifying of factors lead to health adverse effect. Generally, GTS was likely to define base on questionnaire and four main kinds of subjective health symptoms, headache, nausea, dizziness, vomiting. For Thai traditional tobacco processing with maintaining tobacco plants particularly with watering activities that farmers may contacting to wet tobacco leaves which almost of them were conducted in the morning or evening that contribute to exposure with nicotine and pesticide also.

Test-mate AChE Cholinesterase Test System (Model 400); The Test-mate AChE was intended for use in the assessment and diagnosis of asymptomatic pesticide poisoning. Most organophosphate or carbamate pesticides inhibit the blood enzymes erythrocyte acetylcholinesterase (AChE) and/or plasma cholinesterase (PChE) (79), (80). The degree of enzyme inhibition was proportional to the extent of exposure. AChE was generally preferred because of its lower biological variability and lack of interferences relative to PChE. Pre-exposure (baseline) measurements of AChE and/or PChE should be obtained to reduce the effect of biological variability (79). The short-term method of treatment was to simply remove the patient from exposure to pesticides.

Interpretation of Results

In AChE mode the photometric analyzer displays the following results:

AChE (erythrocyte cholinesterase) in U/mL (units per milliliter),

AChE in %N (percent normal) relative to 4.71 U/mL,

Hgb (hemoglobin) in g/dL (grams per deciliter),

Hgb in %N relative to 15.0 g/dL,

Q (quotient) in U/g (units per gram),

Q in %N relative to 31.4 U/g.

Q is a hemoglobin corrected value of erythrocyte cholinesterase. Q was computed by dividing the AChE result by the Hgb result. Should the hemoglobin level be below 5 g/dL, the Q is not calculated.

In PChE mode the photometric analyzer displays the following results:

PChE (plasma cholinesterase) in U/mL,

PChE in %N relative to 2.55 U/mL,

Hgb in g/dL,

Hgb in %N relative to 15.0 g/dL.

Depression of cholinesterase to <50% normal indicates possible pesticide poisoning requiring removal from exposure and/or treatment with anticholinergic such as atropine and ralidoxime (79). Suspected cases of poisoning can be confirmed by cholinesterase monitoring for a subsequent rise and plateau of activity 1 - 3 months after exposure. If baseline values are obtained, depression of cholinesterase to <70% of baseline can be taken to indicate possible pesticide poisoning (81).

2.8 Related Research

In Thailand, (82) study the composition of tobacco dust, atmospheric nicotine concentration, urinary cotinine excretion and the subjective symptoms of workers in dry tobacco leaf preparation and analyzed by GC/MS. It was found that the tobacco dust contained nicotine and atrazine (a herbicide). The average atmospheric nicotine was 0.105 mg/m^3 and urinary cotinine concentrations of post tobacco curing process workers was 3.084 microgram/ml. Moreover, there was a significant correlation between the atmospheric nicotine dust and urinary cotinine excretion (r = 0.987, $p < 0.05$). The health symptoms of headache, nausea, weakness, dizziness, and increased perspiration reported among workers had a significant relationship with the job characteristics of the post tobacco curing process workers, with a p-value < 0.05. Nicotine dust contained a herbicide called atrazine. Nicotine concentrations were highest in the post tobacco curing process where workers reported a lot of adverse symptoms. Urinary cotinine can be used as a biomarker of tobacco dusts' exposure in dry tobacco leave preparation areas. According to the previous study (83). It was found that dermal hand wipes of residual nicotine dust samples, morning urine samples and subjective symptoms were collected from 30 workers. The hand-wipe samples and urine samples were analyzed for nicotine and cotinine by a GC/MS, respectively. The average amount of nicotine on the hands of workers was 0.24 microgram/cm^2, while the average urinary cotinine concentration of workers was 3.08 microgram/ml. Moreover, there was a significant correlation between nicotine residue on hands and urinary cotinine excretion at r = 0.978, $p < 0.05$. There was also a significant relationship between the occupational related nicotine residue on hands and the number of subjective symptoms reported ($p < 0.05$).The nicotine residue on hands could be used as an indicator of occupational nicotine dust exposure which might affect the health of tobacco workers.

CHAPTER III RESEARCH METHODOLOGY

3.1 Research design

Analytical Cross-sectional Study and Prospective Study

3.2 Study population

Phase I: The total population who growing Thai traditional tobacco of Praputthabath Sub-District, Chiangklang District and Phatow Sub-District, Thawangpha District, Nan Province. Farmers and their family members from a representative sample of households in the two select Sub-Districts were the study population were 473 farmers for investigate prevalence of GTS and personal protective behaviors. Of 84 farmers who met definition of GTS were test by Test mate kit for measured AChE and PChE levels.

Phase II: A prospective study was conducted with twenty of Thai traditional tobacco farmers and twenty persons of non-tobacco farmers in two Sub-districts, the subjects were male and female between 20 and 65 years of age a total of 40 participants were randomly selected by drawing technique from tobacco farmers in this area.

The inclusion criteria are male and female between 20 and 65 years of age and general good health ,growing tobacco, harvesting tobacco leaves, or producing dry tobacco regularly season period, local agriculturalist who live in study area (permanent stay), no fever or common cold symptoms , no diarrhea complications.

The exclusion criteria were history of liver or skin disease, drug or alcohol abuse, and use of medication that might be influence nicotine absorption (e.g., nitrates) or nicotine metabolism (e.g., barbiturates).

Exposure protocol subjects were what they normally to work with tobacco cultivation and dry tobacco production. Subjects began working at around 6 A.M. and continued until finished, which was following a 1-2 hr. lunch break.

3.3 Sample size

3.3.1 Phase I Cross-sectional Study

All Thai Traditional Tobacco Farmers who were to participate and registered at Praputthabath Health Promoting Hospital and Phatow Health Promoting Hospital, Nan Province in season 2012/2013. The sample size estimation was calculated by using the following formula

$$n = \frac{Z_{\alpha/2}^{2} P(1-P)}{d^{2}}$$

Where, n= the estimated sample size

Zα/2 = the value from normal distribution associated with confidence interval =1.96 for 95%CI.

α = the level of statistical significance was set as 0.05

p = The proportion of Thai Traditional Tobacco Farmers, the value of 0.75% was determined from survey data of Traditional Tobacco Farmers in Chiangklang Agriculture District and value of 0.90% is determine from survey data of Traditional Tobacco Farmers in Thawangpha Agriculture District, Nan Province (February, 2012)

d = the absolute precision required on either side of proportion of the study, the value of 5% was selected.

Then, we calculated sample size of Praputthabath Sub-District's farmer when Zα/2=1.96, p=0.75, and d=0.05 as

$$n = \frac{(1.96)^2 \times (0.75) \times (0.25)}{(0.05)^2} = 288$$

Therefore, 288 (~290) and with estimate 10% of 290 (or 29) participants will not participate. Therefore, 319 Thai Traditional Tobacco Farmers of Praputthabath Sub-District were required for this study.

We calculated sample size of Phatow Sub-District's Farmer when $Z\alpha/2=1.96$, $p=0.90$, and $d=0.05$ as

$$n = \frac{(1.96)^2 \times (0.90) \times (0.10)}{(0.05)^2} = 138$$

Therefore, 138 (~140) and with estimate 10% of 140 (or 14) participants will not participate. Therefore, 154 Thai Traditional Tobacco Farmers of Phatow Sub-District are required for this study.

Total samples size for these studies are 473 persons (Praputtabath Sub-District 319 persons and Phatow Sub-District 154 persons)

3.4 Sampling method

3.4.1 Phase I Cross-sectional Study

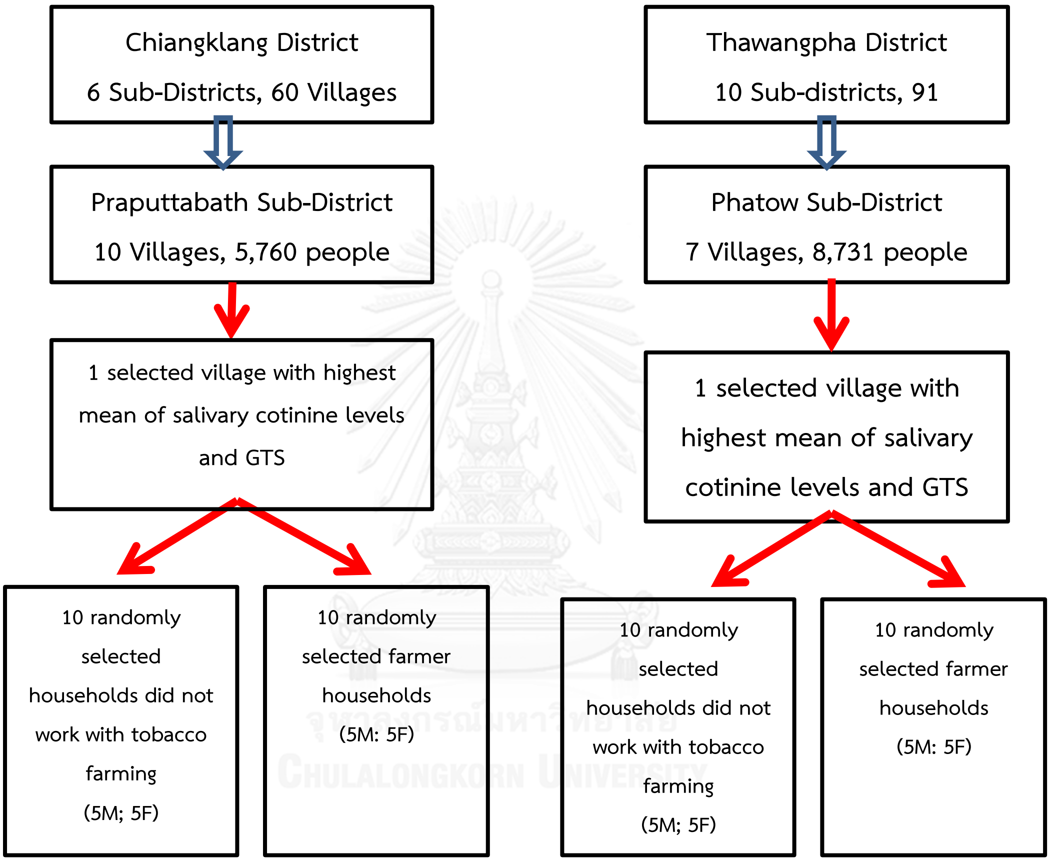
An initial random sample of 10 villages in Praputthabath Sub-District, Chiangklang District and 7 Villages in Phatow Sub-District, Thawangpha District, Nan Province was selected from list of such villages maintain by Health Promoting Sub-District Hospital. Total sample 473 persons(Praputthabath Sub-District 319 persons and Phatow Sub-District 154 persons) were distribute by proportion for each village and Systematic Random Sampling in each village for select a participants and simple random sampling 30% for Salivary cotinine Test with detail in table as below

Table 6 Distribution of sample size for survey and Salivary Cotinine Test

No.Village	Praputthabath Sub-District			No.Village	Phatow Sub-District		
	Households	n	Salivary Test		Households	n	Salivary Test
1	154	28	8	1	72	10	3
2	203	37	11	2	167	23	7
3	103	19	6	3	176	24	7
4	122	22	7	4	70	10	3
5	216	39	12	5	152	21	6
6	251	46	14	6	233	32	10
7	35	6	2	7	246	34	10
8	236	43	13				
9	192	35	11				
10	243	44	13				
Sum	1755	319	96		1116	154	46

3.4.2 Phase II Prospective study, Repeated Cross-sectional Study (comparison between group exposure and non-exposure)

From Phase I, we selected a village who farmer had the mean of Salivary cotinine levels in high level and simple random sampling for amount 10 persons in each Sub-District for salivary cotinine test repeated measure where the farmers were similar occupations and similar living conditions by matching gender and duration year of tobacco farming of exposure group and 10 persons who did not contact with tobacco leaves as control group both were follow-up with questionnaire.



3.5 Research instruments and measurements

Part I Questionnaire

A data collection instrument i.e. questionnaire were used to interview farmer who growing, harvesting and producing dry tobacco from traditional tobacco type by face to face. The Questionnaire was modified based on Arcury and Quandt (27), (46) and with reference to previous studies that are;

BIQ 1 (Individual questionnaire)

For collecting information on individual characteristics at beginning of the study period include:

Part 1 Socio-demographics

There are questions in this part were included gender, age, education levels, and monthly income, occupation, land owned, health problem, experience in tobacco cultivation, etc.

Part 2 Dermal exposure to Nicotine and Personal protective behaviors

There were nine questions in this part. The questions was focused on about the contact with tobacco (Type of work, season), Skin area exposed (work with no long shirt), Surface area to volume ratio; Body Mass index (BMI), Protective clothing (wear rain suit), Dry conditions (change out of wet clothes), Work experience (year worked in tobacco).

Part 3 Transdermal absorption and Personal protective behaviors

This part include the Tobacco use (Tobacco use, live with a smoker) Skin integrity (rashes, cuts, abrasion), Alcohol consumption, Wet conditions (work in wet clothes).

Part 4 Self-reported Health Symptoms and Pesticide exposure levels

Health problem were based on the advice from experts and results of the pilot study include; Headache, nausea, vomiting, weakness, dizziness, runny eyes, blurred vision, increased perspiration and increased salivation (2), (3), (20), (27), (28).

Part 5 salivary cotinine levels by NicAlert^{TM} Saliva strip and Pesticide exposure by Test-mate AChE Cholinesterase Test System (Model 400)

Follow-up individual questionnaire)

For collecting information on each individual during each follow-up round (Biweekly): Tobacco Farm Activities and salivary cotinine levels as measured by NicAlert^{TM} Saliva strip.

Part 1: Dermal exposure to nicotine.

There were seven questions in this part. The questions were focused on about the Contact with tobacco (Type of work, season), Skin area exposed (work with no long shirt), Surface area to volume ratio; Body Mass index (BMI), Protective clothing (wear rain suit), Dry conditions (change out of wet clothes), Work experience (year worked in tobacco).

Part 2: Transdermal absorption

There were seven questions in this part include the Tobacco use (Tobacco use, live with a smoker) Skin integrity (rashes, cuts, abrasion), Alcohol consumption, Wet conditions (work in wet clothes), Humidity (average high humidity), Heat (average mean temperature) (45), (46).

Part 3: Self-reported Health Symptoms

There were nine questions in this part. Health problem were based on the advice from experts and results of the pilot study include; Headache, nausea, vomiting, weakness, dizziness, runny eyes, blurred vision, increased perspiration and increased salivation (2), (3), (20), (27), (28).

Part 4: salivary cotinine levels by NicAlert^{TM} Saliva strip

The levels of salivary Cotinine levels by NicAlert^{TM} Saliva strip on the Tobacco farmers and non-farmers at that time.

Quality control for data collection by questionnaire: Filled questionnaires were submitted by the surveyors to the team leader on daily basis and feedback on the data collected from the team leaders to they were given during the next time. Two staffs from Chiangklang District Hospital, Thawangpha District Hospital and Sub-District Health Promoting Hospital were involved as field supervisor. Monthly review meetings with the participations of the research team, district supervisor, data collection team conducted every month to discuss and solved problems arises during the field work.

3.6 Study procedure

Table 7 Study procedure

Phase I **(Cultivation and maintaining tobacco plants)**		**Phase II** **(Harvesting and Dry tobacco producing)**				
Group	Test	**Group** **(40)**	Salivary cotinine levels by NicAlert™ Saliva strip test and Subjective health symptoms			
473 for cross-sectional study	- Salivary cotinine levels by NicAlert™ Saliva strip and -Personal protective behaviors - Subjective health symptoms		M1	M2	M3	M4 (1 month after finish harvesting and tobacco producing)
		Farmers (20)	X X	X X	X X	X
84 Thai traditional tobacco farmers	Pesticide exposure levels by Test –Mate OP kit - Subjective health symptoms	Non-farmers (20)	X X	X X	X X	X

X: Salivary Cotinine test (Monthly and Biweekly)

3.6.1 Phase I

3.6.1.1 Prevalence of GTS

Materials and Methods

Sample and sampling method

Thai traditional tobacco is cultivated in Nan Province, a province in the northern region of Thailand composed of 15 districts. Almost all districts have been involved in the cultivation of traditional tobacco for 60 years. Five districts are devoted to tobacco cultivation with two of them being large-scale areas—Thawangpha and Chiangklang Districts. Seven villages and 1,116 households in

Phatow Sub-District in Thawangpha District and ten villages and 1,755 households in the Praputthabath Sub-District in Chiangklang District were chosen as the population and study area. The household representatives were responsible for completing interviewer-administration questionnaires. The calculated sample size was 473 subjects from a total of 2,871 farmers, both male and female, between 20 and 65 years of age. The subjects were recruited by means of systematic random sampling from tobacco farmers in the area. The inclusion criteria were farmers who were in generally good health and they were growing tobacco, picking tobacco leaves, or producing dry tobacco regularly during the season. On the other hand, the exclusion criteria were farmers who did not exhibit any particular symptoms from either tobacco sickness or from pesticide exposure.

Measurement tool

A cross-sectional study was conducted with 473 Thai traditional tobacco farmers in Praputtabath Sub-District and Phatow Sub-District. The farmers were randomly selected and interviewed in an in-person survey that had been modified from the previous study (11), (12), (46). The interview questionnaire was validated by a panel of three experts to ensure its content validity, and the Index of Objective Congruence (IOC) was equal to 0.87. After that, the interview questionnaire was tried out with 30 subjects whose demographic characteristics were similar to those of the subjects of the main study to determine its reliability, and Cronbach's alpha coefficient was 0.81. The items contained in the survey questions elicited data regarding demographic characteristics (gender, age, family status, level of education, current smoking status, and alcohol consumption), work-related conditions (process of tobacco plant cultivation with seeding, growing, watering, fertilizing, and using pesticides), process of maintaining tobacco plants (watering, cutting top and axillaries buds, dropping herbicide on the top and buds, fertilizing, removing grass, and spraying insecticide), and picking and curing tobacco leaves (picking tobacco leaves, transporting leaves from farm to home, and curing tobacco leaves). The process of dry tobacco production consists of removing the stem of tobacco leaves, rolling a bundle of tobacco leaves, cutting tobacco leaves with a cutting machine, laying slices of tobacco on a bamboo rack, drying tobacco slices in a rack in the sunlight, flipping a bamboo rack, spraying tobacco extract to adjust tobacco color, packing dry tobacco in a plastic bag, and picking a bamboo rack. The interview questionnaire also elicited data regarding GTS, which referred to subjective health symptoms that gave rise to acute nicotine poisoning caused by dermal absorption of nicotine from the mature tobacco plants. GTS symptoms included vomiting, nausea, headaches, and dizziness (11) . The in-person survey and environmental survey were undertaken in December 2012.

Data Analysis

All data were coded and entered in to the Statistical Package for Social Sciences (SPSS) version 17. Statistical analyses were conducted, and descriptive statistics of frequency, percentage, mean, and standard deviation were used. In addition, the prevalence of GTS was stratified by farmers' characteristics, work-related characteristics, and subjective health symptoms following the definition. Moreover, inferential statistics of Chi-square test and Fisher's exact test were utilized to determine the relationship between the characteristics and GTS. A multiple logistic regression was also employed to explore risk factors of GTS, with 95% confidence interval (95% CI) of odds ratio (OR). Furthermore, the variables that were associated with GTS at the level of 0.20 from the univariate analysis were selected into the multiple logistic regression models. The final model included gender, smoking, skin integrity, working with a wet suit, working in the curing process, and watering tobacco plants. The likelihood Ratio test (LRT) was used to test the variables associated with GTS by comparing models with and without the referring variables. All variable levels were coded so that the reference level (OR = 1) represented the hypothetical advantageous level concerning increased GTS. Finally, the Wald test was performed to test the significance of each level compared with the reference level on particular variables. All tests were tested with the significance level set at 0.05.

3.6.1.2 GTS and Pesticide exposure levels

MATERIAL AND METHODS

Subjects and sampling

The sample of the present study was Thai traditional tobacco farmers in Phatow sub-district, Thawangpha district, and Praputthabath sub-district, Chiangklang district in Nan province, Thailand. The farmers in the selected sub-districts who met the classification criteria of GTS in our previous study were identified, totaling 107 persons. The sample size for this study was then calculated with 95% of confidence interval. In the end, a total of 84 tobacco farmers were recruited, 40 of whom were from Phatow sub-district and 44 from Praputthabath sub-district. The formula used in sample size calculation was as follows:

$$n = \frac{N}{1+N(e)^2}$$

n = sample size

N = population of GTS farmer (N=107), e = 0.05

All the study participants were randomly recruited with a drawing technique from the population of farmers living in the selected sub-districts. It is worth noting that all of the participants gave their informed consent before participating in the study. As regards their demographic characteristics, all of them were involved in pesticide application during the process of Thai traditional tobacco cultivation. They were between 25 and 60 years of age. Before data collection commenced, the study participants underwent a physical examination by nurse practitioners working at a health promoting hospital in the areas. The participants' medical records were obtained as well.

Measurement tool

The present study was cross-sectional research which was conducted with 84 randomly selected Thai traditional tobacco farmers involved in pesticide application during the process of tobacco cultivation. During data collection, the farmers were interviewed using a face-to-face questionnaire that had been adapted from a previous study undertaken by Arcury et al. (2002). It is noteworthy that although there are no firmly established diagnostic criteria for GTS, Arcury et al. had developed a clinically useful case definition of GTS based on symptoms and susceptible working conditions. The identified symptoms of GTS include headache, nausea, dizziness, and vomiting reported after a day of working with tobacco (84). Data regarding demographic characteristics of the study participants elicited in this study included gender, age, family status, level of education, current smoking status, and alcohol consumption, while data regarding work-related conditions gathered from the participants included process of tobacco seedling and planting involving seedling, planting, watering, fertilizing, and applying pesticides for protection of the roots of the tobacco plants. Other data collected involved process of maintaining tobacco plants including watering, cutting the top and axillary buds, dropping a herbicide on the top and buds, fertilizing, getting rid of grass and weeds, and spraying insecticide; picking and curing tobacco leaves including picking tobacco leaves, transferring the leaves from the farm to home, and curing tobacco leaves; dry tobacco producing including removing the stem of tobacco leaves, rolling a bundle of tobacco leaves, cutting tobacco leaves with a cutting machine, putting a slice of tobacco on a bamboo rack, drying the rack of tobacco leaves in the sunlight, reversing the bamboo rack, spraying a tobacco extract to adjust the tobacco color, packing dry tobacco in a plastic bag, and putting away the bamboo rack. The face-to-face questionnaire, the environmental survey, and the measurements of the blood AChE and PChE levels with the Test-Mate OP Kit (EQM Inc., Junefield, Ohio) were administered in December 2012. This field assay used a modified Ellman method (85) and received extensive field and lab testings (80, 86-88)AChE activity, measured as absorbance, was corrected for ambient temperature and hemoglobin. Based on the assumption that GTS symptoms may occur only with significant lowering of AChE level in the blood (89), the measurement of the frequency adverse health effects in a population may depend more strongly on the proportion on very low AChE levels than on those whose levels represent the population mean. To account for this possibility, cholinesterase levels were dichotomized to normal and risk categories as well as treated on a continuous scale (90). The finger-prick blood of the participants was collected with a capillary tube and the procedure described in the manual of the Test-Mate OP kit was strictly followed. The AChE levels were further grouped into two groups. The values less than 2.7 U/ml were interpreted as 'risky,' while those ≥ 2.7 U/ml as 'safe.' In addition, the PChE values less than 1.3 U/ml were interpreted as 'risky,' while those ≥ 1.3 U/ml as 'safe.' The cut-off points for these categories were stated in the manual.

Data Analysis

All data were coded and entered into the Statistical Package for Social Sciences (SPSS) software version 17. In the first phase of the analysis, statistical analyses were conducted using frequency and percentage to describe qualitative data, whereas mean and standard deviation were used to describe quantitative data. Thai traditional tobacco farmers who met the classification criteria of GTS were considered having indicators of the potential for pesticide exposure, and AChE and PChE levels were examined among these farmers. In the second phase of the analysis, the symptoms associated with pesticide exposure were identified. Pesticide exposure was defined by both low AChE levels and a report of exposure during the Thai traditional tobacco cultivation and production process, including use of pesticides. For each of the symptoms, odd ratios were used to estimate the ratio of observed to expected cases. Chi-square and Fisher's exact test were also employed to find out if there was any association between the symptoms of GTS and AChE levels. In this study, it was assumed that GTS symptoms were associated with AChE levels because GTS and nicotine poisoning inhibit neurotransmitters similar to a reduction in AChE levels. Also, both GTS and low AChE levels result in similar symptoms including headache, nausea, and vomiting. Finally, adjusted odd ratios were estimated by means of logistic regression analysis. All results were determined to be significant at $P < 0.05$ using 95% confidence interval.

3.6.1.3 GTS and Personal protective behaviors

Sample and sampling method

This study was conducted in December 2012. The target population of this study was the farmers who cultivated Thai traditional tobacco plants in Nan province, a province in the northern region of Thailand. Seven villages and 1,116 households in Phatow sub-District in Thawangpha district and ten villages and 1,755 households in Praputthabath sub-district in Chiangklang district were chosen as the target population and research settings. The representatives of households in the chosen sub-districts were randomly chosen with a drawing technique and were asked to respond to the questionnaire. There were both male and female subjects who ranged in age from 20 to 65 years of age, totaling 473 tobacco farmers. They were local tobacco farmers who were in a generally good health, and they grew tobacco for tobacco leaves or produced dry tobacco leaves during the season. In addition, they had no fever or other symptoms of a common cold, no diarrhea, and no subjects exposure to pesticides applied in their tobacco farm at the time of data collection because the pesticide was used when tobacco plants was early cultivation and this interview conducted after that around three months .

Measurement tool

A cross-sectional study was conducted with 473 Thai traditional tobacco farmers in Praputtabath sub-district and Phatow sub-district. The farmers were randomly selected and interviewed using a face-to-face questionnaire that was modified from the instrument used in a previous study (33) and an environmental survey. Data collected included individual characteristics of the farmers (gender, age, family status, level of education, current smoking status, and alcohol consumption), work related conditions (process of tobacco plantation and cultivation: seedling, growing, watering, fertilizing; pesticide use for protection of roots of tobacco plants, insecticide application when tobacco plants was young; process of maintaining tobacco plants (watering, cutting the top and axillaries buds, dropping herbicide on the top and buds, fertilizing, getting rid of grass, spraying insecticide); and picking and curing tobacco leaves (picking tobacco leaves, transferring them from farm to home, and curing tobacco leaves). Other data collected included the process of drying tobacco (removing the stem of tobacco leaves, rolling a bundle of tobacco leaves, cutting tobacco leaves with the cutting machine, putting a slice of tobacco on a bamboo rack, bringing a rack of tobacco out to dry in the sunlight, flipping the bamboo rack, spraying a tobacco extract to adjust the tobacco color, packing dry tobacco in a plastic bag, and picking a bamboo rack). In this study, GTS refers to subjective health symptoms that are caused by acute nicotine poisoning due to dermal absorption of nicotine from mature tobacco plants within two to three days, with typical symptoms including vomiting, nausea, headache, and dizziness that followed the definition of a previous study (11). General information including farming description, handling of tobacco leaves, and use of personal protective equipment (PPE) was also elicited from the subjects and observed by the researchers and well-trained interviewees. The acute symptoms consistent with the definition of GTS were asked in the form of dichotomous (YES/NO) outcomes.

Data Analysis

All data were coded and entered in to the Statistical Package for Social Sciences (SPSS) version 17 (licensed for Chulalongkorn University). Statistical analyses were conducted using frequency and percentage to describe qualitative data, while mean and standard deviation were used with quantitative data. The prevalence of GTS were stratified based on farmers' characteristics, work related characteristics, and subjective health symptoms following the definition. Finally, the association between GTS and use of PPE was analyzed by means of Chi-square test.

3.6.2 Phase II Prospective study and Repeated Cross-sectional Study (comparison between group exposure and control)

3.6.2.1 GTS and Salivary Cotinine levels

The subjects examine are 20 male and 20 female who met all the inclusion criteria and cultivating, harvesting Thai traditional tobacco in Praputthabath Sub-District, Chiangklang District and Phatow Sub-District, Thawangpha District, Nan Province.

Sample size calculation

$$n = \frac{Z^2_{\alpha/2} P(1-P)}{d^2}$$

Z= 1.645 P= 0.82, d= 0.1

$$n = \frac{(1.645)^2 (0.82)(0.18)}{(0.1)^2}$$

$$n = 39.85; \sim 40$$

Where, n= the estimated sample size

$Z\alpha/2$ = the value from normal distribution associated with confidence interval =1.645 for 99%CI.

α = the level of statistical significance is set as 0.01

p = The proportion of subjective health symptoms with nicotine poisoning (0.82%) (83)

d = the absolute precision required on either side of proportion of the study, the value of 1% was selected.

MATERIAL AND METHODS

Sample and sampling method

A prospective study was conducted with 20 Thai traditional tobacco farmers and 20 non-tobacco farmers in Praputtabath Sub-District and Phatow Sub-District in Nan Province, totaling 40 subjects. There were both male and female subjects who ranged in age from 20 to 65 years old. The subjects were randomly selected by means of a drawing technique from tobacco farmers in this area. They were then classified into two groups—the cases and the controls. As for the former, they were Thai traditional tobacco farmers who picked tobacco leaves or produced dried tobacco during a regular season, while the latter consisted of non-tobacco farmers who lived in the same area as the cases. The subjects, who were local agriculturists living in the area, were generally in good health, had no fever or other symptoms of a common cold, no diarrhea complications, and no exposure to pesticides.

Measurement tool

The farmers were randomly classified into two groups of Thai traditional tobacco farmers and non-tobacco farmers. Data were collected by means of interviews using a face-to-face questionnaire that had been modified from the instrument used in a previous study by Arcury et al. (2002) and an environmental survey. The questionnaire elicited data regarding demographic characteristics of the subjects (gender, age, family status, level of education, current smoking status, and history of alcohol consumption); their working conditions; and the process of dried tobacco production consisting of picking tobacco leaves, transferring leaves from farm to home, curing tobacco leaves, removing the stem of tobacco leaves, rolling a bundle of tobacco leaves, cutting tobacco leaves with a cutting machine, putting slices of tobacco leaves on a bamboo rack, bringing the rack of tobacco to dry in the sun, reversing the bamboo rack, spraying a tobacco extract to adjust tobacco color, packing dried tobacco in a plastic bag, and putting away the bamboo rack. Other data gathered from the subjects included use of personal protective equipment (PPE) and number of hours working in dried tobacco production. Once again, In this study, GTS referred to subjective health symptoms caused by acute nicotine poisoning due to dermal absorption of nicotine in mature tobacco plants. Its symptoms included vomiting, nausea, headaches, and dizziness (11). Salivary samples were collected at each contact so as to measure cotinine concentration levels using the NicAlert™ saliva strip test or NCTS. The participants were randomly selected and interviewed using face to face questionnaires with bi-weekly follow-up for 7 times and at each contact to measuring cotinine concentration by NicAlert™ Saliva strip tests; NCTS.

Salivary cotinine evaluation

The salivary cotinine level was evaluated using the NicAlert™ Saliva strip tests (Nymox Pharmaceutical Cooperation, St.-Laurent, QC, Canada). The system provides a semi-quantitative measure of cotinine in saliva for the purpose of determining whether an individual has been exposed to tobacco products within the previous 48 hours. The NicAlert™ saliva strip zones range from level 0 (0-10 ng/mL, non-user of tobacco products) to level 6 (> 1000 ng/mL, user of tobacco products). The cut-off concentration for the NicAlert™ saliva strip (an immunochromatographic assay using monoclonal antibody), indicating a positive results, is 10 ng/mL (zones 1-6). The salivary cotinine concentration and the interpretation for each level of the NicAlert™ saliva strip test are shown in Table 6 below (91). Salivary cotinine levels were recorded after squeezing eight drops from the saliva-containing tubes (after bringing it to room temperature) directly onto the white padded end of the strip. Results were read after allowing the strip to develop by laying it on the marked area of the plastic laminated instruction card for 15 to 30 minutes. The lowest numbered zone displaying a red color was documented as the NicAlert^{TM} saliva strip test result (91).

Table 8 cotinine concentration and its interpretation for each level of the NicAlert^{TM} test

Level	**Cotinine concentration (ng/mL)**	**Interpretation**
0	0-10	Non -user of tobacco products
1	10-30	User of tobacco products
2	30-100	User of tobacco products
3	100-200	User of tobacco products
4	200-500	User of tobacco products
5	500-1000	User of tobacco products
6	>1000	User of tobacco products

Salivary cotinine level was records after squeezing eight drops from the saliva-containing tubes (after bringing it to room temperature) directly onto the white padded end of the strip. Results were read after allowing the strip to develop by laying it on the marked area of the plastic laminated instruction card for 15 to 30 minutes. The lowest numbered zone displaying a red color was documented as the NicAlert^{TM} Saliva strip test result (91).

Salivary cotinine data collection

The interview follow-ups were conducted bi-weekly from 08:00 a.m. to 07:00 p.m. each time, from December 2012 to March 2013, totaling seven visits (twice in November, twice in December, twice in January, and once in March). Ten trained interviewers conducted the interviews with selected groups of Thai traditional tobacco farmers and non-tobacco farmers. The interviews were conducted two times during the monthly surveys (bi-weekly), and one time as a follow-up after the farmers had finished their tobacco work of the season. During the interviews, data regarding the occurrence of subjective health symptoms and risk factors of nicotine exposure were collected, included smoking status, process of tobacco work, use of personal protective equipment (PPE), and number of hours working in dried tobacco production. Besides this, salivary samples were collected during each contact with the subjects so as to measure cotinine concentration levels using the NCTS.

Data analysis

All data collected from the study subjects were coded and entered into the Statistical Package for Social Sciences (SPSS) version 17. Descriptive statistics of frequency and percentage were used to analyze qualitative data and mean and standard deviation were utilized to analyze quantitative data. The data regarding prevalence of GTS as stratified by farmers' characteristics during each contact, dried tobacco production, and subjective health symptoms were analyzed following previously identified definitions. Furthermore, a correlation between GTS, dried tobacco production process, PPE use, and salivary cotinine levels was analyzed by means of Spearman's correlation (Spearman's rho) with significance levels of both 0.01 and 0.05.

Table 9 Describe the study process and corresponding farming activities in the two sites.

Time	Study process	Farming activities	
Aug-12	**-**	Seeding tobacco	Watering/Fertilizing feeding/Insecticide spray
Sep-12	-	Transplanting and growing Maintain tobacco plants	Watering/Fertilizing feeding/Insecticide spray
Oct/Nov-12	**Phase I;** questionnaire /Salivary Cotinine test/ pesticide exposure levels	Maintain tobacco plants	Watering /Fertilizing feeding/Insecticide spray /Watering/ Insecticide spray/Topping (Cutting top of tobacco plants)/Removing axillary buds/Drop herbicide for control top and axillary buds of tobacco plants
Dec-12	**Phase II; M1; (Biweekly)** Questionnaire+ salivary cotinine test)	Dry tobacco producing	Watering/ Removing axillary buds/ Picking tobacco leaves-Picking tobacco leaves/Transporting tobacco leaves/Curing tobacco leaves/Removing stem ripen tobacco leaves/Cutting tobacco leaves/Putting a tobacco slice on the bamboo plate/Reverse-bamboo plate/Adjust color of tobacco slices/Fold dry tobacco slices and packaging
Jan-13	**Follow-up1; M2 (Biweekly)** (Questionnaire+ salivary cotinine test)	Dry tobacco producing	
Feb-13	**Follow-up2 ;M3 (Biweekly)** (Questionnaire+ salivary cotinine test)	Dry tobacco producing	

Table 10 Describe the study process and corresponding farming activities in the two sites. (Cont.)

Time	Study process	Farming activities	
Mar-13	**M4;** (Questionnaire+ salivary cotinine test)	1 month after finish harvesting and tobacco producing (normal cotinine levels in body without working with tobacco)	No activities with tobacco farming

3.7 Ethical Considerations

This study was approved by Ethical consideration from the College of Public Health Sciences, Chulalongkorn University COA No.170/2555.

CHAPTER IV RESULTS

4.1 GTS and Prevalence

Of the 473 subjects, 50.7% were male and 49.3% were female. The average age (± SD) of the subjects was 51.82 (± 7.39) years. More than half of the subjects (56.7%) were the head of their family, and most of them, or 83.1%, completed primary education and 15.8% completed secondary education. Almost all of the study subjects had been working on a traditional tobacco plantation for more than 20 years, and their smoking rate was less than 12%. In terms of alcohol consumption, almost two-thirds of the subjects, or 62%, drank alcohol. In addition, the prevalence of GTS was 22.62% (95% CI = 19.08-26.60). When stratified by personal and work-related characteristics, the prevalence rates of GTS were 17.92% (95% CI = 13.58-23.26) and 27.47% (95% CI = 22.14-33.53) between males and females, respectively (Table 11, 12).

Table 11 Characteristic and Prevalence of GTS on Thai traditional tobacco farmers (n=473)

Characteristics 9 (n=473)	**n**	**%**
Gender		
Male	240	50.7
Female	233	49.3
Age group (years)		
35 - 51	248	52.4
52 - 65	225	47.6
Mean = 51.82; S.D.= 7.39; Min = 35; Max = 65		

Table 12 Characteristic and Prevalence of GTS on Thai traditional tobacco farmers (n=473) (Cont.)

Characteristics 9 (n=473)	n	%
Status in family		
Head of family	268	56.7
Housewife	195	41.2
Member	10	2.1
Education level		
Primary school	393	83.1
Secondary school	75	15.8
Higher than secondary school	5	1.1
Smoking		
No	420	88.8
Yes	53	11.2
Alcohol consumption		
No	182	38.5
Yes	291	61.5
Experience with tobacco plantation (years)		
< 20	52	11.0
≥ 20	421	89.0
Current work with tobacco (hours per day)		
≤5	199	42.1
6-10	274	57.9
Mean = 5.26; S.D.= 4.19; Min = 0; Max = 10		
Prevalence of GTS	473 (107)	22.62, 95%CI (19.08-26.60)
Male Total (n)	240 (43)	17.92, 95%CI (13.58-23.26)
Female Total (n)	233 (64)	27.47, 95%CI (22.14-33.53)

There was a significant difference of GTS by gender ($p = 0.013$) (Table 13). Also, there was a statistically significant difference in GTS between workers with more than 20 years of experience with tobacco plantation and those with more or less than 20 years ($p = 0.017$). Furthermore, a statistically significant difference was also found in the subjects who worked with tobacco in the plantation on the day of the interview ($p = 0.001$). However, there was no statistically significant difference in GTS ($p = 0.279$) in the farmers who removed the tops of the plants, even though cutting axillaries buds of tobacco plants and watering tobacco plants resulted in statistically significant difference in GTS ($p = 0.028$ and $p = 0.001$, respectively). With regard to use of protective gears, the findings showed that there was no statistically significant difference in GTS between the subjects who wore a long-sleeved shirt, long pants, a rain coat, a plastic apron, gloves and boots compared to those who did not have such protective gears ($p > 0.05$. Furthermore, there was a statistically significant difference in GTS of the subjects who worked with a wet suit and those who washed their hands with acid soap compared to those who did not have such protection, at $p = 0.081$ and $p = 0.021$,respectively. Besides this, a statistically significant association was found in GTS and most of the farmers who worked in a wet suit while watering tobacco plants who did not change their clothes until they finished their work. By the same token, groups of workers who worked with a wet suit as well as those who did not wash their hands with acid soap had a higher prevalence rate of GTS.

Table 13 Association of Green Tobacco Sickness (GTS) by demographic and work-related characteristics among Thai traditional tobacco farmers (n=473)

Factors	Green Tobacco Sickness (GTS) n (%)		OR	95% CI	*p*-value*
	No	Yes			
Gender					
Male	197(53.8)	43(40.2)	1.73	1.12-2.68	0.013*
Female	169(46.2)	64(59.8)			
Education level					
Primary school	302 (82.5)	91(85.0)	0.83	0.45-1.50	0.539
Secondary school and higher	64(61.9)	16(18.1)			
Smoking					
No	319(87.2)	101(94.4)	0.40	0.16-0.97	0.037*
Yes	47(12.8)	6(5.6)			
Living with smokers					
No	342(93.4)	97(90.7)	0.14	0.67-3.17	0.326
Yes	24(6.6)	10(9.3)			
Alcohol consumption					
No	119(32.5)	63(58.9)	0.33	0.21-0.52	0.001*
Yes	247(67.5)	44(41.1)			
Skin rash					
No	121(33.1)	16(15.0)	2.80	1.58-4.98	0.001*
Yes	245(66.9)	91(85.0)			

Table 13 Association of Green Tobacco Sickness (GTS) by demographic and work-related characteristics among Thai traditional tobacco farmers (n = 473) (Cont.)

Factors	**Green Tobacco Sickness (GTS) n (%)**		**OR**	**95% CI**	***p*-value***
	No	**Yes**			
Experience with tobacco plantation (years)					
< 20	47(18.2)	5(4.7)	3.00	1.16-7.76	0.017*
≥ 20	319(87.2)	102(95.3)			
Current work with tobacco					
No	33(9.0)	0 (0)	1.32	1.25-1.39	0.001*
Yes	333(91.0)	107(99.5)			
Cutting top of tobacco plants					
No	282(77.0)	77(72.0)	1.30	0.80-2.12	0.279
Yes	84(23.0)	30(28.0)			
Cutting axillaries buds of tobacco plants					
No	241(65.8)	58(54.2)	1.62	1.05-2.52	0.028*
Yes	125(34.2)	49(45.8)			
Watering tobacco plants					
No	290(79.2)	62(57.9)	2.77	1.74-4.38	0.001*
Yes	76(20.8)	45(42.1)			

Table 13 Association of Green Tobacco Sickness (GTS) by demographic and work-related characteristics among Thai traditional tobacco farmers (n = 473) (Cont.)

Factors	Green Tobacco Sickness (GTS) n (%)		OR	95% CI	*p*-value*
	No	Yes			
Curing tobacco leaves					
No	359(98.1)	83(77.6)	2.77	1.74-4.38	0.001*
Yes	7(1.9)	24(22.4)			
Wearing a long-sleeved shirt					
No	69(18.9)	18(16.8)	1.14	0.64-2.03	0.634
Yes	297(81.1)	89(83.2)			
Wearing long pants					
No	43(11.7)	9(8.4)	1.45	0.68-3.07	0.332
Yes	323(88.3)	98(91.6)			
Wearing a raincoat					
No	221(60.4)	73(68.2)	0.71	0.44-1.12	0.141
Yes	145(39.6)	34(31.8)			
Wearing a plastic apron					
No	240(65.6)	72(67.3)	0.92	0.58-1.46	0.742
Yes	126(34.4)	35(32.7)			
Wearing gloves					
No	214(58.5)	60(56.1)	1.10	0.71-1.70	0.659
Yes	152(41.5)	47(43.9)			

Table 13 Association of Green Tobacco Sickness (GTS) by demographic and work-related characteristics among Thai traditional tobacco farmers (n = 473) (Cont.)

Factors	Green Tobacco Sickness (GTS) n (%)		OR	95% CI	*p*-value*
	No	Yes			
Wearing boots					
No	202(55.2)	59(55.1)	1.00	0.65-1.54	0.993
Yes	164(44.8)	48(44.9)			
Wearing wet suit					
No	147(40.2)	33(30.8)	0.66	0.41-1.05	0.081*
Yes	219(59.8)	74(69.2)			
Hand washing with acid soap					
No	95(26.0)	40(37.4)	0.58	0.37-0.92	0.021*
Yes	271(74.0)	67(62.6)			

* The p-value was based on the Chi-square test or Fisher's Exact test.

Finally, multiple logistic regression analysis (Table 14) shows the risk factors significantly associated with GTS after being adjusted for gender, smoking, skin integrity, wearing a wet suit, type of work with tobacco in a day including curing tobacco leaves and watering tobacco plants which were analyzed in terms of gender (OR_{adj} = 0.44, 95% CI = 0.26-0.73), smoking (OR_{adj} = 4.36, 95% CI = 1.41-13.47), skin rash (OR_{adj} = 0.36, 95% CI = 0.19-0.68), wearing a wet suit (OR_{adj} = 1.91, 95% CI = 1.12-3.23), process of curing tobacco leaves, (OR_{adj} = 0.06, 95% CI = 0.02-0.16), and watering tobacco plants (OR_{adj} = 0.42, 95% CI = 0.25-0.72).

Table 14 Multivariate logistic regression analysis of risk factors of Green Tobacco Sickness (GTS) among Thai traditional tobacco farmers (n=473)

Factors	**Green Tobacco Sickness (GTS) n (%)**		**OR (95% CI)**	**OR $_{adj}$ (95% CI)**	***p*-value***
	No	**Yes**			
Gender					
Male	197 (53.8)	43 (40.2)	1.73 (1.12-2.68)	0.44 (0.26-0.73)	0.002*
Female	169 (46.2)	64 (59.8)			
Smoking					
No	319 (87.2)	101 (94.4)	0.40 (0.16-0.97)	4.36 (1.41-13.47)	0.011*
Yes	47 (12.8)	6 (5.6)			
Skin rash					
No	121 (33.1)	16 (15.0)	2.80 (1.58-4.98)	0.36 (0.19-0.68)	0.001*
Yes	245 (66.9)	91 (85.0)			
Watering tobacco plants					
No	290 (79.2)	62 (57.9)	2.77 (1.74-4.38)	0.42 (0.25-0.72)	0.002*
Yes	76 (20.8)	45 (42.1)			
Curing tobacco leaves					
No	359 (98.1)	83 (77.6)	2.77 (1.74-4.38)	0.06 (0.02-0.16)	0.001*
Yes	7 (1.9)	24 (22.4)			

Table 14 Multivariate logistic regression analysis of risk factors of Green Tobacco Sickness (GTS) among Thai traditional tobacco farmers (n=473) (Cont.)

Factors	**Green Tobacco Sickness (GTS) n (%)**		**OR (95% CI)**	**OR $_{adj}$ (95% CI)**	*p*-value*
	No	**Yes**			
Wearing a wet suit					
No	147 (40.2)	33 (30.8)	0.66 (0.41-1.05)	1.91 (1.12-3.23)	0.016*
Yes	219 (59.8)	74 (69.2)			

* The p-value was based on the Wald Chi-square test.

4.2 GTS and Pesticide exposure levels

There were 84 Thai traditional tobacco farmers with GTS who participated in the present study. Their blood AChE and PChE levels were measured and data were gathered by means of the questionnaire interview. Of the 84 study participants, 45.2% were male and 54.8% were female. The mean age of the participants was 46.6 ± 6.5 years old (with the range of 25 to 60 years old). Furthermore, more than half of the participants (52.4%) were housewives, and more than three quarters (78.6%) completed only primary education. As regards their work experience, almost all of them (97.6%) had been working with Thai traditional tobacco plantation for over 20 years, and nearly half of them (46.4%) worked with tobacco around six to ten hours per day. As regards history of cigarette smoking, almost all of them (96.4%) were non-smokers, but 7.1% were living with someone who smoked. Finally, nearly half of the participants (46.4%) were alcohol consumers. The results regarding demographic characteristics of the study sample are summarized in Table 15, 16 below.

Table 15 Demographic characteristics of Thai traditional tobacco farmers with GTS (n = 84)

Demographic characteristics	**Number (n)**	**Percentage (%)**
Gender		
Male	38	45.2
Female	46	54.8
Age (years)		
25 - 46	36	42.9
47 - 60	48	57.1
Status in family		
Head of family	40	47.6
housewife	44	52.4
Education levels		
Primary education	66	78.6
Secondary education	18	41.4

Table 16 Demographic characteristics of Thai traditional tobacco farmers with GTS (n = 84) (Cont.)

Demographic characteristics	Number (n)	Percentage (%)
History of cigarette smoking		
No	80	95.2
Yes	4	4.8
Alcohol consumption		
No	45	53.6
Yes	39	46.4
Experience with tobacco plantation (years)		
< 20	2	2.4
≥ 20	82	97.6
Approximated daily tobacco exposure (hours)		
≤5	45	53.6
6-10	39	46.4

As shown in Table 17 below, the results revealed that the arithmetic mean value of AChE was 2.7 ± 0.6 U/ml (Min = 1.8; Max = 4.4 U/ml). The mean value of PChE was 1.9 ± 0.4 U/ml (Min = 1.1; Max = 2.9 U/ml). The cut-off point for these categories with PChE value less than 1.35 U/ml. was interpreted as 'risky' and PChE value ≥ 1.35 U/ml. as 'safe.'

Table 17 Acetylcholinesterase and plasmacholinesterase values among Thai traditional tobacco farmers with GTS. (N=84)

GTS farmers	**Acetylcholinesterase (U/ml.)**	**Plasmacholinesterase (U/ml.)**
Mean	2.72	1.91
Std. Deviation	0.643	0.451
Minimum	1.81	1.12
Maximum	4.46	2.97

According to Table 18 below, almost two-thirds of the farmers who participated in this study (61.9%) had their AChE at a risky level, while more than one-third (38.1%) had their AChE at a safe level. The results showed that the symptoms caused by use of pesticides that were similar to symptoms of GTS were nausea (7.1%) and vomiting (8.3%). Also, it was found that the prevalence of common pesticide symptoms such as nausea or vomiting among the Thai traditional tobacco farmers whose AChE levels were at a safe level were significantly higher than those of the farmers whose AChE levels were at a risky level ($p < 0.05$).

Table 18 Association between AChE levels and symptoms of GTS among Thai traditional tobacco farmers (n = 84)

Symptoms of GTS	**Acetylcholinesterase levels n (%)**		**Total (%) (n = 84)**	**OR**	**95% CI**	*p*-value[a]
	Safe[b] (n = 32)	**Risky[b] (n = 52)**				
Headache	24(75.0)	42(80.8)	66(78.6)	1.40	0.48-4.02	0.111
Nausea	6(18.8)	0(0)	6(7.1)	0.33	0.24-0.45	*0.002**
Dizziness	16(50.0)	16(30.8)	32(38.1)	0.44	0.17-1.10	0.078
Vomiting	6(18.8)	1(1.9)	7(8.3)	0.09	0.01-0.74	*0.011**
Weakness	31(96.9)	47(90.4)	78(92.9)	0.30	0.03-2.72	0.262
Running eyes	6(18.8)	10(19.2)	16(19.0)	1.03	0.33-3.17	0.957
Blurred vision	0(0)	3(5.8)	3(3.6)	0.60	0.50-0.72	0.166
Increased sweating	8(25.0)	13(25.0)	21(25.0)	1.00	0.36-2.76	1.00
Increased saliva	0(0)	5(9.6)	5(6.0)	0.59	0.49-0.71	0.084

[a]: The p-value were based on the Chi-square test or Fisher's Exact test <0.05.

[b]: A acetylcholinesterase value < 2.77 U/ml. was interpreted 'risky' and ≥ 2.77 U/ml. 'safe'.

With regard to the PChE levels among the study participants, the study results revealed that 42.9% of the participants had their PChE at a risky level, whereas 57.1% had their PChE at a safe level. In addition, the results showed that the common pesticide symptoms that were related to symptoms of GTS were headache (78.6%), dizziness (38.1%), and increased saliva (6.0%). The prevalence of common pesticide symptoms such as headache and increased saliva among the Thai traditional tobacco farmers with PChE at a risky level were significantly higher than those of the farmers with PChE at a safe level ($p < 0.05$). On the other hand, the symptom of dizziness experienced by the farmers with PChE at a safe level was significantly higher than that of the farmers whose PChE was at a risky level ($p < 0.05$), (Table 19).

Table 19 Association between PChE levels and symptoms of GTS among Thai traditional tobacco farmers (n = 84)

Symptoms of GTS	**Plasmacholinesterase levels n (%)**		**Total (%) n = 84**	**OR**	**95% CI**	***p*-value**[a]
	Safe[b] **n = 48**	**Risky**[b] **n = 36**				
Headache	34(70.8)	32(88.9)	66(78.6)	3.29	0.98-11.06	*0.046**
Nausea	4(8.3)	2(5.6)	6(7.1)	0.64	0.11-3.74	0.696
Dizziness	23(47.9)	9(25.0)	32(38.1)	0.36	0.14-0.93	*0.032**
Vomiting	4(8.3)	3(8.3)	7(8.3)	1.0	0.20-4.77	1.00
Weakness	43(89.6)	35(97.2)	78(92.9)	4.07	0.45-36.47	0.179
Running eyes	10(20.8)	6(16.7)	16(19.0)	0.76	0.24-2.32	0.630
Blurred vision	1(2.1)	2(5.6)	3(3.6)	2.76	0.24-31.74	0.574
Increased sweating	12(25.0)	9(25.0)	21(25.0)	1.00	0.36-2.71	1.00
Increased saliva	0(0)	5(13.9)	5(6.0)	0.39	0.29-0.51	*0.012**

[a] :The p-value were based on the Chi-square test or Fisher's Exact test <0.05.

[b] :A plasmacholinesterase value < 1.35 U/ml. was interpreted 'risky' and ≥ 1.35 U/ml. 'safe'.

Table 20 Association between AChE levels and PChE levels among Thai traditional tobacco farmers with GTS (n = 84)

Markers	Plasmacholinesterase levels n (%)		Total (%)	OR	95% CI	*p*-value*
	Safe	Risky				
Acetylcholinesterase levels						
safe	20(41.7)	12(33.3)	32(38.1)	1.42	0.58-3.51	0.436*
Risky	28 (58.3)	24(66.7)	52(61.9)			
Total	48(100)	36(100)	84(100)			

*p>0.05

Based on the study results, there was no statistically significant difference between AChE levels and PChE levels ($p > 0.05$) (See Table19). Moreover, in the multivariate analysis (See Table 18), two variables with $p < 0.05$ in the bivariate analyses were simultaneously analyzed with logistic regression analysis. The results showed that only one variable was significantly associated with AChE levels after being adjusted for the vomiting symptom (OR_{adj} = 11.76, 95% CI = 1.34-102.98) (See Table 21).

Table 21 Logistic regression analysis of acetylcholinesterase (AChE) levels by symptoms of Green Tobacco Sickness (GTS) among Thai traditional tobacco farmers (n = 84)

Symptoms	**Acetylcholinesterase level n (%)**		**OR(95% CI)**	**OR $_{adj}$(95% CI)**	***p*-value***
	Safe n = 32	**Risky n = 52**			
Vomiting					
No	26(81.2)	51(98.1)	0.09 (0.01,0.74)	11.76(1.34,102.98)	0.026*
Yes	6(18.8)	1(1.9)			

* The p-value <0.05

According to the multivariate analysis (Table 18), three variables with $p < 0.05$ in the bivariate analyses were simultaneously analyzed using logistic regression analysis. The results revealed that only one variable was statistically significantly associated with PChE levels after being adjusted for the dizziness symptom (ORadj = 2.76, 95% CI = 1.07-7.08) (See Table 22).

Table 22 Logistic regression analysis of plasmacholinesterase (PChE) levels by symptoms of Green Tobacco Sickness (GTS) among Thai traditional tobacco farmers (n = 84)

Symptoms	**Plasmacholinesterase level n (%)**		**OR (95% CI)**	**OR $_{adj}$(95% CI)**	***p*-value***
	Safe n = 48	**Risky n = 36**			
Dizziness					
No	25(52.1)	27(75.0)	0.36(0.14,0.93)	2.76(1.07,7.08)	0.035*
Yes	23(47.9)	9(25.0)			

* The p-value <0.05

4.3 GTS and Personal protective behaviors

There were 473 Thai traditional tobacco farmers who participated in the questionnaire interview. As regards demographic characteristics of the farmers, half of them, or 50.7%, were male, while 49.3% were female. In terms of age, they ranged in age from 35 to 65 years old, with the average of age of 51.82 years old (SD = 7.39). Moreover, half of the subjects, or 56.7%, were head of the family. As for educational background, the majority, or 83.1%, completed primary education, and 15.8% completed secondary education. In addition, most of them, or 89%, had experiences with Thai traditional tobacco plantation for more than 20 years, and 57.9% worked with tobacco around six to ten hours per day. With regard to history of smoking, 88.8% of the farmers did not smoke cigarettes, but nearly two-thirds, or 61.5%, had history of alcohol consumption. The findings regarding demographic characteristics of the study subjects are summarized in Table 23, 24 below.

Table 23 Demographic characteristics of the study sample (n = 473)

Characteristics (n = 473)	**N**	**%**
Gender		
Male	240	50.7
Female	233	49.3
Age group (years)		
35 - 51	248	52.4
52 - 65	225	47.6
Mean = 51.82; SD = 7.39; Min = 35; Max = 65		
Status in family		
Head of family	268	56.7
Housewife	195	41.2
Member	10	2.1
Education level		
Primary school	393	83.1
Secondary school	75	15.8
Higher than secondary school	5	1.1

Table 24 Demographic characteristics of the study sample (n = 473) (Cont.)

Characteristics (n = 473)	**N**	**%**
History of cigarette smoking		
No	420	88.8
Yes	53	11.2
History of alcohol consumption		
No	182	38.5
Yes	291	61.5
Experience with tobacco plantation (years)		
< 20	52	11.0
≥ 20	421	89.0
Number of hours working with tobacco per day		
0-5	199	42.1
6-10	274	57.9
Mean = 5.26; SD = 4.19; Min = 0; Max = 10		

Table 25 presents the frequency of personal protective equipment (PPE) usage during work with tobacco processing. Most of the subjects reported that they always used PPE (long sleeved shirt, long legged pants, and mask). However, 67.7%

and 65.8% of the subjects had never worn a rain coat or a plastic apron, respectively. In addition, even though a mask was always used by almost two-thirds of the subjects (62.4%), it could be seen that more than one-fourth of the subjects, or 28.5%, had never used it. Finally, nearly two-thirds of the subjects, or 65.5%, never changed their wet suit clothes after finishing their work with tobacco plants.

Table 25 Use of personal protective equipments among Thai traditional tobacco farmers (n=473)

Use of PPE	Thai traditional tobacco farmers: n (%)		
	Never	Sometimes	Always
Long sleeved shirts	88(18.6)	7(1.5)	378(79.9)
Long legged pants	52(11.0)	1(0.2)	420(88.8)
Rain coat	320(67.7)	80(16.9)	73(15.4)
Plastic apron	311(65.8)	94(19.9)	68(14.4)
Gloves	274(57.9)	41(8.7)	158(33.4)
Boots	271(57.3)	118(24.9)	84(17.8)
Mask	135(28.5)	43(9.1)	295(62.4)
Changing wet clothes after work	310(65.5)	94(19.9)	69(14.6)

The association between green tobacco sickness (GTS) and personal protective equipment was found as the farmers who wore a plastic apron or mask had a statistically significant association with GTS ($p = 0.001$ and $p = 0.044$, respectively). However, wearing a long-sleeved shirt, long-legged pants, rain coat, gloves, and boots were not statistically significantly associated with GTS ($p > 0.05$). Moreover, a good practice of changing wet clothes after work was strongly statistically significantly associated with GTS ($p = 0.001$), (Table 26).

Table 26 the association between the use of PPE and Green Tobacco Sickness (N=473)

PPE vs. GTS	χ^2	*P*-value
Long sleeved shirts vs. GTS	2.194	0.344
Long legged pants vs. GTS	1.253	0.535
Rain coat vs. GTS	1.885	0.390
Plastic apron vs. GTS	13.125	<0.001**
Gloves vs. GTS	0.499	0.779
Boots vs. GTS	0.993	0.609
Mask vs. GTS	6.234	0.044*
Changing wet clothes after work vs. GTS	14.500	<0.001**

The p-value were based on the Chi-square test or Fisher's Exact test <0.05.

*Significant at 0.05 probability level

**Significant at 0.01 probability level

4. 4. GTS and Salivary cotinine levels

There were 40 subjects who participated in the questionnaire interviews. In terms of their demographic characteristics, the subjects ranged in age from 42 to 60 years old. The average age (±S.D.) of the subjects was 50.18 (±4.93) years old. With regard to gender, 50.0% were male and 50.0% were female. More than half of the subjects (55.0%) were the head of the family, and most of them (85.0%) completed primary education only. In addition, almost two-thirds of them (60.0%) worked with tobacco around six to ten hours each day. Only one subject, or 5%, was current cigarette smoker, and all of them, or 100%, had never lived with anyone who smoked. Also, only 10.0% of the subjects drank alcohol. Finally, almost two-thirds of the subjects, or 65.0%, had normal body mass index (BMI). The findings regarding demographic characteristics of the study sample are summarized in Table 27, 28.

Table 27 Demographic characteristic of the study sample (n=40)

Demographic characteristics	Nontobacco farmers (n = 20)	Tobacco farmers (n = 20)
Gender		
Male	10 (50.0)	10 (50.0)
Female	10 (50.0)	10 (50.0)
Age (years)		
42 - 50	11(55.0)	11(55.0)
51 - 60	9(45.0)	9(45.0)
(Mean = 50.18; SD = 4.93; Min = 42; Max = 60)		
Status in family		
Head of family	10(50.0)	11(55.0)
housewife	10(50.0)	9(45.0)
Education level		
Primary education	20(100)	17(85.0)
Secondary education	0(0)	3(15.0)

Table 28 Demographic characteristic of the study sample (n=40) (Cont.)

Demographic characteristics	**Nontobacco farmers (n = 20)**	**Tobacco farmers (n = 20)**
Cigarette smoking		
No	20(100)	19(95.0)
Yes	0(0)	1(5.0)
Alcohol consumption		
No	19(95.0)	18(90.0)
Yes	1(5.0)	2(10.0)
Total hours of daily work with tobacco		
0-5	0(0)	8(40.0)
6-10	0(0)	12(60.0)
(Mean = 5.26; SD = 4.19; Min = 0; Max = 10)		

All of testing in seven times, the correlation of salivary cotinine levels on Thai traditional tobacco farmers was different between non-tobacco farmers and tobacco farmers ($p<0.05$). In the test of T1, T2, T3, T4, T5, T6 and T7 was found that in each time of testing, tobacco farmers group have a numbers of salivary cotinine exposure more than non- farmer that measured by NCTS strip test. **Test 1:** totally of testing found on Level 0 (0-10 ng/mL of Cotinine Concentration) were twenty five persons

(62.5%); farmer groups were seven persons (35.0%) and non- farmer group were eighteen persons (90.0%). Total of testing found on Level 1 (10-30 ng/mL of Cotinine Concentration) was seven persons (17.5%); farmers group were five persons (25.0%) and non- farmer group were two persons (10.0%). Total of testing found on Level 2 (30-100 ng/mL of Cotinine Concentration) was five persons; farmers group were five persons (25.0%) and none of non- farmer group. **Test 2:** totally of testing found on Level 0 (0-10 ng/mL of Cotinine Concentration) were twenty seventeen persons (42.5%); farmer groups were four persons (20.0%) and non- farmer group were thirteen persons (65.0%). Total of testing found on Level 1 (10-30 ng/mL of Cotinine Concentration) were two persons (5.0%); farmers group was one person (5.0%) and non- farmer group was one person (5.0%). Total of testing found on Level 2 (30-100 ng/mL of Cotinine Concentration) were ten persons (25%); farmers group were five persons (25.0%) and five persons (25.0%) of non- farmer group. Total of testing found on Level 3 (100-200 ng/mL of Cotinine Concentration) were eleven persons (27.5%); farmers group were ten persons (50.0%) and one persons (5.0%) of non- farmer group. **Test 3**: totally of testing found on Level 0 (0-10 ng/mL of Cotinine Concentration) were thirteen persons (32.5%); farmer group was one person (5.0%) and non- farmer group were twelve persons (50.0%). Total of testing found on Level 1 (10-30 ng/mL of Cotinine Concentration) were six persons (15.0%); farmers group were three person (15.0%) and non- farmer group were three persons (15.0%). Total of testing found on Level 2 (30-100 ng/mL of Cotinine Concentration) were eleven persons (27.5%); farmers group were six persons (30.0%) and five persons (25.0%) of non- farmer group. Total of testing found on Level 3 (100-200 ng/mL of Cotinine Concentration) were nine persons (22.5%); farmers group were nine persons (45.0%) and none of non- farmer group. Total of testing found on Level 4 (200-500 ng/mL of Cotinine Concentration) was one person (2.5%); farmers group was one person (5.0%) and none of non- farmer group. **Test 4**: totally of testing found on Level 0 (0-10 ng/mL of Cotinine Concentration) were sixteen persons (40.0%); farmer group were three persons (15.0%) and non- farmer group were thirteen persons (65.0%). Total of testing found on Level 1 (10-30 ng/mL of Cotinine Concentration) were two persons (5.0%); none of farmers group and non- farmer group were two persons (10.0%). Total of testing found on Level 2 (30-100 ng/mL of Cotinine Concentration) were six persons (15.0%); farmers group were three persons (15.0%) and three persons (15.0%) of non- farmer group. Total of testing found on Level 3 (100-200 ng/mL of Cotinine Concentration) were thirteen persons (32.5%); farmers group were eleven persons (55.0%) and two of non- farmer group (10.0%). Total of testing found on Level 4 (200-500 ng/mL of Cotinine Concentration) were three persons (7.5%); farmers group were three persons (15.0%) and none of non- farmer group. **Test 5**: totally of testing found on Level 0 (0-10 ng/mL of Cotinine Concentration) were eighteen persons (45.0%); farmer group were five persons (25.0%) and non- farmer group were thirteen persons (65.0%). Total of testing found on Level 1 (10-30 ng/mL of Cotinine Concentration) were nine persons (22.5%); three persons (15.0%) of farmers group and non- farmer group were six persons (30.0%). Total of testing found on Level 2 (30-100 ng/mL of Cotinine Concentration) were seven persons (17.5%); farmers group were six persons (30.0%) and one person (5.0%) of non- farmer group. Total of testing found on Level 3 (100-200 ng/mL of Cotinine Concentration) were five persons (12.5%); farmers group were five persons (25.0%) and none of non- farmer group. Total of testing found on Level 4 (200-500 ng/mL of Cotinine Concentration) was one person (2.5%); farmers group was one person (5.0%) and none of non- farmer group. **Test 6**: totally of testing found on Level 0 (0-10 ng/mL of Cotinine Concentration) were seventeen persons (42.5%); none of farmer group and non- farmer group were seventeen persons (85.0%). Total of testing found on Level 1 (10-30 ng/mL of Cotinine Concentration) were six persons (15.0%); three persons (15.0%) of farmers group and non- farmer group were three persons (15.0%).

Total of testing found on Level 2 (30-100 ng/mL of Cotinine Concentration) were six persons (15.0%); farmers group were six persons (30.0%) and none of non- farmer group. Total of testing found on Level 3 (100-200 ng/mL of Cotinine Concentration) were eleven persons (27.5%); farmers group were eleven persons (55.0%) and none of non- farmer group. **Test 7**: totally of testing found on Level 0 (0-10 ng/mL of Cotinine Concentration) were thirty-nine persons (97.5%); nineteen persons (95.0%) of farmer group and non- farmer group were twenty persons (100.0%). Total of testing found on Level 1 (10-30 ng/mL of Cotinine Concentration) was one person (2.5%); one person (5.0%) of farmers group and none of non- farmer group. The results of the test of salivary cotinine levels was found that almost of farmer group have higher levels of cotinine concentration more than non-farmer group (Table 29).

Table 29: Distribution of salivary cotinine levels in Thai traditional tobacco farmers and non-farmers by times of testing (n = 40)

Level	Cotinine concentration (ng/mL)	T1* n (%)			T2* n (%)			T3* n (%)			T4* n (%)		
		NF, n = 20	F, n = 20	Total, n = 40	NF, n = 20	F, n = 20	Total, n = 40	NF, n = 20	F, n = 20	Total, n = 40	NF, n = 20	F , n = 20	Total, n = 40
0	0-10	18(90.0)	7(35.0)	25(62.5)	13(65.0)	4(20.0)	17(42.5)	12(60.0)	1(5.0)	13(32.5)	13(65.0)	3(15.0)	16(40.0)
1	10-30	2 (10.0)	5 (25.0)	7 (17.5)	1(5.0)	1(5.0)	2(5.0)	3(15.0)	3(15.0)	6(15.0)	2(10.0)	0(0)	2(5.0)
2	30-100	0 (0)	5 (25.0)	5(12.5)	5(25.0)	5(25.0)	10(25.0)	5(25.0)	6(30.0)	11(27.5)	3(15.0)	3(15.0)	6(15.0)
3	100-200	0 (0)	3 (15.0)	3 (7.5)	1(5.0)	10(50.0)	11(27.5)	0(0)	9(45.0)	9(22.5)	2(10.0)	11(55.0)	13(32.5)
4	200-500	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(5.0)	1(2.5)	0(0)	3(15.0)	3(7.5)

Level	Cotinine concentration (ng/mL)	T5* n (%)			T6* n (%)			T7* n (%)		
		NF, n = 20	F, n = 20	Total, n = 40	NF, n = 20	F, n = 20	Total, n = 40	NF, n = 20	F, n = 20	Total, n = 40
0	0-10	13(65.0)	5(25.0)	18(45.0)	17(85.0)	0(0)	17(42.5)	20(100.0)	19(95.0)	39(97.5)
1	10-30	6(30.0)	3(15.0)	9(22.5)	3(15.0)	3(15.0)	6(15.0)	0(0)	1(5.0)	1(2.5)
2	30-100	1(5.0)	6(30.0)	7(17.5)	0(0)	6(30.00	6(15.0)	0(0)	0(0)	0(0)
3	100-200	0(0)	5(25.0)	5(12.5)	0(0)	11(55.0)	11(27.5)	0(0)	0(0)	0(0)
4	200-500	0(0)	1(5.0)	1(2.5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

*P < 0.05, T = time of testing; T7 = testing done one month after tobacco work finished, NF = Non-farmers, F = Farmers

The results of the tests indicated that there was a correlation between tobacco farmers and salivary cotinine levels at every testing, except for the seventh test that did not reveal such a correlation ($p > 0.05$). The strong correlation between dried Thai traditional tobacco production process and salivary cotinine levels was likely to result from the tobacco farmers' involvement in various kinds of work including picking tobacco leaves, grading tobacco leaves, curing, removing the stem of tobacco leaves, rolling a bundle, cutting leaves, putting tobacco slices on a bamboo rack, drying tobacco slices on the rack, and packaging dried tobacco leaves ($p < 0.01$) (See Table 30, 31).

Table 30 the correlation between dried tobacco production process and salivary cotinine levels among Thai traditional tobacco farmers and non-farmers (n = 40)

Dried tobacco production process	Salivary cotinine levels (R)						
	T1	T2	T3	T4	T5	T6	T7***
Tobacco Farmers (n = 20)	0.591**	0.538**	0.680**	0.631**	0.539**	0.894**	0.160
Picking tobacco leaves	0.249	0.391*	0.680**	0.641**	0.539**	0.631**	NA
Transferring tobacco leaves	0.361*	0.244	0.311	0.476**	0.396*	0.435**	NA
Grading tobacco leaves	0.415**	0.402*	0.720**	0.414**	0.474**	0.433**	NA
Curing tobacco leaves	0.436**	0.324*	0.545**	0.371*	0.319*	0.303	NA

Table 31 The correlation between dried tobacco production process and salivary cotinine levels among Thai traditional tobacco farmers and non-farmers (n = 40) (Cont.)

Dried tobacco production process	Salivary cotinine levels (R)						
	T1	T2	T3	T4	T5	T6	T7***
Removing the stem of tobacco leaves	0.525**	0.458**	0.616**	0.631**	0.397*	0.433**	NA
Rolling bundles of tobacco leaves	0.429**	0.508**	0.616**	0.631**	0.397*	0.420**	NA
Cutting tobacco leaves	0.238	0.448**	0.465**	0.538**	0.273	0.420**	NA
Putting tobacco slices on a bamboo rack	0.526**	0.203	0.355*	0.512**	0.159	0.518**	NA
Reversing the bamboo rack	0.404**	0.513**	0.477**	0.364*	0.417**	0.523**	NA
Spraying a tobacco extract	0.133	0.369*	0.231	0.121	NA	NA	NA
Keeping dried tobacco	0.214	0.419**	0.553**	0.744**	0.487**	0.620**	NA

*Significant at 0.05 probability level, **Significant at 0.01 probability level, ***T7 = Control (one month after finishing tobacco work)

According to the study findings, the correlation between four main symptoms of green tobacco sickness (GTS) including headache, nausea, vomiting, and dizziness and salivary cotinine levels were found. Simply put, tobacco farmers were likely to have a strong correlation with salivary cotinine levels as shown in six out of the seven tests conducted (T1-T6). Furthermore, it was found that headache was correlated with salivary cotinine levels at every test. On the other hand, vomiting was found to be correlated with salivary cotinine levels in three tests (T4, T5, and T6), whereas nausea was not found to have a correlation with salivary cotinine levels in all six tests (T1-T6). Finally, dizziness was strongly correlated with salivary cotinine levels only in the first test (T1). The correlation between tobacco farmers' use of personal protective equipment and salivary cotinine levels was highest in the sixth test. The correlation between PPE use of wearing a long-sleeved shirt, wearing gloves, and wearing a face mask was found to be high in all of the first six tests (T1-T6) with the p-value of 0.01 (See Table 32, 33).

Table 32 the correlation between use of personal protective equipment (PPE) and salivary cotinine levels among Thai traditional tobacco farmers and non-farmers (n = 40)

PPE use in dried tobacco production process	Salivary cotinine levels (R)						
	T1	T2	T3	T4	T5	T6	T7***
Tobacco Farmers (n =20)	0.591**	0.860**	0.680**	0.631**	0.539**	0.894**	0.160
Wearing a long-sleeved shirt	0.442**	0.692**	0.575**	0.471**	0.529**	0.494**	NA
Wearing long pants	0.549**	1.000	0.427**	0.342*	0.511**	0.510**	NA
Wearing a raincoat	-0.120	0.186	NA	NA	NA	NA	NA
Wearing a plastic apron	0.021	0.489**	0.368*	0.259	0.379*	0.304	NA

Table 33 The correlation between use of personal protective equipment (PPE) and salivary cotinine levels among Thai traditional tobacco farmers and non-farmers (n = 40) (Cont.)

PPE use in dried tobacco production process	Salivary cotinine levels (R)						
	T1	T2	T3	T4	T5	T6	T7***
Wearing gloves	0.411**	0.692**	0.635**	0.631**	0.559**	0.690**	NA
Wearing boots	0.233	0.603**	0.349*	0.553**	0.575**	0.631**	NA
Wearing a face mask	0.591**	0.860**	0.680**	0.631**	0.539**	0.894**	NA
Changing a wet suit during work	0.034	0.440**	0.196	0.333*	0.261	0.496**	NA

*Significant at 0.05 probability level, **Significant at 0.01 probability level, ***T7 = Control (one month after finishing tobacco work)

The correlation between GTS experienced by tobacco farmers and salivary cotinine levels was found in all of the tests ($p < 0.01$). However, it is worth noting that the prevalence of GTS was found to increase in an earlier test (T2) before subsequently declining during T3 to T5. At T7, the prevalence of GTS was found to be equal to 10%, which meant that only one of the tobacco farmers who also smoked cigarettes had symptoms that met the definition of GTS. At T7, a negative correlation between GTS and salivary cotinine levels was found (See Table 34)

Table 34 the correlation between GTS and salivary cotinine levels among Thai traditional tobacco farmers and non-farmers (n = 40)

GTS in dried tobacco production process	Salivary cotinine levels						
	T1	T2	T3	T4	T5	T6	T7
Green Tobacco Sickness (n = 20)	R = 0.740**	R = 0.451**	R = 0.485**	R = 0.675**	R = 0.641**	R = 0.735**	R = -0.053
Prevalence of GTS (95%CI)	32.5 (20.08, 47.98)	57.5 (42.20, 71.49)	55.0 (39.83, 69.29)	52.5 (37.50, 67.06)	45.0 (30.71, 60.17)	47.5 (32.94, 62.50)	10.0 (3.96, 23.05)

** Correlation was significant at the 0.01 level (2-tailed); T= time to testing

CHAPTER V DISCUSSIONS

5.1 GTS and Prevalence

Based on the study findings, it could be seen that the occurrence of GTS in females was 1.73 higher than that males. Almost all farmers graduated from a primary school and farming was their traditional vocation. This is consistent with the fact that Thai traditional tobacco cultivation in this area is a part of local culture and folk life. It is worth noting that even though the health effects of tobacco cultivation were known to these farmers, they did not recognize the route of effects or the causes of known health problems. Such a finding yielded support to the finding of a previous study (2). In addition, the results of this study showed that the total prevalence rate of GTS was 22.62%. This can be considered the first documentation of GTS prevalence among Thai traditional tobacco farmers in Nan Province. In fact, there is a wide range of prevalence of GTS in the literature, from 8% to 89% per season. The highest prevalence report may be due to unspecific case definitions (Gosh et al., 1979) (92). Two studies found very high relative odds of GTS when farmers were working in wet conditions.(46), (26) Moreover, the prevalence rate in males was 17.92% and that in females was 27.47%. This finding was inconsistent with what has previously been reported in other international studies that almost all of the farmers affected by GTS were male (84). In traditional tobacco cultivation in Thailand, female farmers share the role of intensive producers through their labor with their male counterparts. (93, 94). Similarly, there was no gender difference in the report of health problems of tobacco farmers in Vietnam.(95) Furthermore, an odds ratio of GTS was 24.04% in farmers who were non-smokers compared to those who were smokers. Such findings were consistent with the differences in GTS in two groups of farmers reported by Gehlbach et al. (5) that farmers believed in the protective effects of tobacco use. In this study, however, famers did not see smoking as having a protective effect for GTS. Only 11% of the farmers were smokers. Thus, work processes associated with GTS were identified. For instance, the work activities that are associated with GTS include exposure to wet plants and to mature tobacco plants, as well as cutting of axillaries buds, which put farmers at risk of excessive exposure to nicotine in the mature plants. This is because farmers have to repeatedly carry out such activities every seven days. To further explain, to remove axillaries, farmers have to walk through and move up and down the rows of plants, hence exposure to nicotine bearing foliage. Moreover, farmers use their hands and arms when working, and they can easily come into contact with the sap and gum from the tobacco plants when transferring the leaves from the pick-up truck or pushcart to the place where tobacco leaves are cured. Also, watering the plants with a hose, sprinkler, or pipeline is associated with GTS via dermal exposure, and GTS is also associated with changing work clothes. Wet clothing may increase exposure to nicotine from mature plants via dermal absorption. As found in other studies, Personal Protective Equipment (PPE) use among farmers can significantly statistically reduce GTS. (20). However, it is noteworthy that Environmental Tobacco Smoke (ETS) exposure through living with smokers does not have a significant effect on GTS. Another issue that should be mentioned here is that many studies of GTS do not attempt to establish the criteria for the diagnosis of GTS. Thus, a direct assessment and diagnosis of GTS, or a biomarker of GTS, should be verified. The measurements that have been used in previous studies are correlated with cotinine levels. (10), (11). Finally, a validated questionnaire is needed to determine if the combined symptoms of headache, dizziness, nausea, or vomiting occurred only among those who have been working in Thai traditional tobacco cultivation or not. (92).

5.2 GTS and Pesticide exposure levels

Based on the study results, it could be concluded that the health effects of tobacco cultivation were known to Thai traditional tobacco farmers; nevertheless, the actual causes of such effects may not have been clearly understood. Such a result was consistent with the result reported by a previous study (2). In addition, the results of this study showed that the prevalence of the risky level of AChE was 61.9% and the prevalence of the safe level was 38.1%. Also, the prevalence of the risky and safe levels of PChE was 42.9% and 57.1%, respectively. Such results indicated that Thai traditional tobacco farmers who were involved in intensive agriculture may have been exposed to pesticides from their activities in the cultivating season because before these farmers started growing tobacco plants in the areas, they had been growing vegetables, sticky rice, and other agricultural products. Besides this, Thai traditional tobacco cultivation always involves use of pesticides (e.g., organophosphate and carbamates) in early stages of the cultivation process in order to protect the roots of the tobacco plants with Carbofuran (Curater®). Also, methomyl, Malathion, or pyrethroids may be sprayed onto the tobacco plants depending on infestation of insects. Also, the present study found that common symptoms of GTS were related to symptoms of pesticide exposure when farmers had AChE at a safe level, including nausea (7.1%) and vomiting (8.3%), while the symptom of GTS that was related to the symptom of pesticide exposure when farmers had PChE at a safe level was dizziness (38.1%). It is noteworthy that these results were different from the results of previous studies (96, 97) that the decrease in AChE and PChE levels was not statistically significantly associated with symptoms of pesticide exposure. However, it may be possible explain such association with the definition of GTS, including any general illness after exposure to tobacco leaves (46) and use of specifically applied case definition of headache, nausea, dizziness, or vomiting during or after exposure to tobacco leaves as nicotine poisoning (11). Furthermore, even when the farmers have a safe level of AChE and PChE, abnormal symptoms may still occur after exposure to a low dose of pesticides exposure and changes in cholinesterase inhibition that may cause similar symptoms with different mechanisms. It is also possible that previously depressed acetylcholinesterase levels may have occurred before enrollment (90). It is also possible that symptoms such as nausea, vomiting, headache, and dizziness may result from organophosphorous insecticide exposure when organophosphorous insecticides are applied (98). However, it is worth noting that chemical pesticides will not be applied during harvest of Thai traditional tobacco plants, and the definition of GTS refers to a group of symptoms that occur after working with tobacco plants without any previous application of pesticides. Therefore, it is possible to interpret that the safe levels of AChE and PChE are associated with nicotine poisoning as well

The health effects of tobacco cultivation were known but not recognize the route of effects or causes of these health problems this finding supports the previous study (2).The results of this study show total the prevalence of risky level of AChE was 61.9% and safe level was 38.1%, risky and safe level group of PChE was 42.9% and 57.1% respectively. From this results indicated that Thai traditional tobacco farmers are intensive agricultural may exposure to pesticide from their activity in crop season because in this area before they growing tobacco they have been growing vegetables, sticky rice, and others (36). Moreover, Thai traditional tobacco cultivation used some of pesticides (Organophosphate and carbamates) in early step for protect roots of tobacco plants with Carbofuran (Curater®) and spraying with methomyl, Malathion or Pyrethroid depend on invasion of insects. The present study found that the most common symptoms of GTS related to pesticide exposure symptoms with safe level of AChE were nausea (7.1%), vomiting (8.3%) and safe level of PChE were dizziness (38.1%). This result different to those of previous studies(96), (97) that the inhibition of AChE and PChE (risk level) not statistically significant association with symptoms of pesticide exposure. However, the association may possible to explain with a define of GTS, as any general illness after exposure to tobacco leaves (46) and use a specific applied case definition was headache or nausea or dizziness or vomiting during or after exposure to tobacco leaves as nicotine poisoning (11). There for safe level of AChE and PChE be able to occurred common symptoms with low dose pesticide exposure and changing exposure to cholinesterase –inhibiting pesticides and others pesticides causing similar symptoms by different mechanism and previously depressed acetylcholinesterase are levels may have occurred before enrollment (90) and symptoms such as nausea, vomiting, headache or dizziness that result from organophosphorous insecticide exposure could also occur when organophosphorous insecticides are applied (98). Even though, Thai traditional pesticide application was less use in harvesting period and with the definition of GTS a symptoms after working with tobacco in which some of them do not use pesticide before, thus possible to interpret that with a safe level of AChE and PChE associated with nicotine poisoning also.

5.3 GTS and personal protective behaviors

Based on the findings of this study, it could be seen that almost all of the farmers worked in wet clothes when watering tobacco plants and never changed clothes until they finished work. Such a practice was found to have a statistically significant association with GTS. In fact, watering tobacco plants needs to be conducted every seven days. In this study, the farmer who watering tobacco plants with a hose, sprinkler, or pipe line may have been associated with GTS. Such findings were consistent with findings of a previous study (10) that a wet condition supports dermal absorption of nicotine. Furthermore, GTS was associated with changing out of wet clothing. In this study, the farmers never changed from their wet clothes (65.5%) until they finished their work of the day or in the evening. Thus, continuously wearing wet clothes may increase exposure to nicotine from mature plants which have more soluble nicotine that can be more easily absorbed via the skin. This helped explain why a wet condition has been found to be related to GTS. Even in dry working condition, the farmers who wear a long-sleeved shirt or long-legged pants may be less susceptible to exposure to nicotine on tobacco leaves. However, if they work in a wet condition, their wet clothes may increase area absorption of nicotine as nicotine is water soluble. Likewise, a previous study has reported similar findings (14). Thai traditional tobacco farmers are engaged in several work processes associated with GTS, as similarly reported by Quandt et al. (10). In this study, it was found that types of work were associated with the exposure to wet plants and to matured tobacco plants that have high nicotine content. In the process to maintain tobacco plants, cutting axillaries buds is related to exposure to nicotine in the mature plants, and the farmers must engage in this process every seven days until the plants are matured. As the leaves or all parts of the plants contain nicotine, and when the farmers break the axillaries buds, they have to use their hands to bring out the buds or twist the buds from the plants. In so doing, their hands will be exposed to the plants' juice or sap. Moreover, in this process, the farmers have to walk through and move up and down rows of plants, which can increase their exposure to nicotine as well. Accordingly, in this study, it was found that there was statistically significant different between the famers who always wore a plastic apron and those who did not use a plastic apron when it came to association with GTS. In addition, during the process of picking tobacco leaves, curing tobacco leaves, and putting tobacco slices on the bamboo rack, the farmers use their hands and arms to contact with the juice and sap of the plants. Similarly, their hands contact the juice and sap of the plants when they transfer the leaves from the pickup truck or the pushcart to the place of curing (air curing) in their home or nearby places. In particular, in the process of putting tobacco slices on the bamboo rack or picking dry tobacco, even though the farmers use a rubber latex glove, it does not protect them from exposure to nicotine on their hands because hot climate may promote sweating on their hands, so the moisture from sweat may still lead to GTS in the farmers. Such findings yielded support to findings of a previous study (83). However, such findings were inconsistent with findings of earlier studies in which a variety of seamless knitted hand gloves were tested to determine prevention of dermal nicotine absorption and nylon gloves were found to be most durable and suitable in all the processes of tobacco cultivation (25) and the use of any type of gloves significantly reduced the levels of nicotine ($p < 0.01$) and cotinine ($p < 0.0005$) in the urine (99). In contrast, in the present study, almost all of the subjects wore rubber latex gloves and reused they until they leaked; thus, nicotine may be absorbed through dermal contact. This may explain why there were no differences in association with GTS between the farmers who used and those who did not use rubber latex gloves. In addition, previous studies have also revealed that wearing boots could reduce nicotine absorption (25, 39). However, in the present study, the farmer used boots and worked with tobacco plants in a dry condition, so wearing boots may not be associated with GTS. Meanwhile, in the watering process, the farmers who did not use boots or gloves during their work in a wet condition manifested symptoms of GTS, and such a finding was consistent with the finding of a previous study (20). Furthermore, it was discovered that the farmers who wore a mask (nose mask) had a negative relationship with GTS; the negative association could be explained with the inverse direction of two factors. In this study, the farmers who worked with dry tobacco processing including putting tobacco slices on the bamboo rack or flipping the bamboo rack and picking dry tobacco had a close contact with the tobacco pieces which were only around two to three feet away in front of them. The farmers sat down and used their hands to handle the situation, and this may lead to inhalation exposure, causing these farmers to be exposed to nicotine dust through both inhalation and dermal contact, hence susceptibility to GTS (19). The personal protective equipment used among farmers in this study has shown to be related to different magnitude of GTS. Put another way, protective equipment could decrease the magnitude of GTS significantly. Moreover, in order to use the equipment, farmers felt that they should be comfortable as well while working in a hot climate (5), (11). Finally, the results of this study were correlated to the results of Gehlbach et al, (5) that has already been mentioned as a key element in primary prevention of GTS/nicotine absorption in tobacco harvesters. Knowledge should be provided on site, to promote hand washing and allow workers time to change if their clothes are soaking wet. In short, in order to prevent health effects, and washing should be provided all over the facilities, and the knowledge should also be made available to all tobacco farmers (11).

5.4 GTS and Salivary cotinine levels

The final product of Thai traditional tobacco cultivation was dried tobacco. Tobacco is mostly cultivated and harvested in the northern region of Thailand. A unique processing method of dried tobacco production was carried out by Thai traditional tobacco farmers, and it required intensive hardworking labors and family labors. The demographic characteristics of the study sample were consistent with the populations living in rural areas in the north of Thailand. Almost all of them graduated from a primary school and farming was the traditional occupation. Moreover, the average of age of these farmers was quite high, as older adults generally make up the largest proportion of agriculturalists at present time in the rural areas of Thailand. Thai traditional tobacco cultivation in this area is a part of local culture and folk life. Previous studies have reported that there are a number of health effects of tobacco cultivation which are caused by nicotine which penetrates through the skin of the hands of workers who cultivate and harvest tobacco (1), (2), (3). In the present study, the prevalence of GTS among farmers was higher in early tests (T1, T2) and declined in subsequent tests (T3 to T5). Such findings yielded support to the study of Trapé-Cardoso, Bracker (100) who found that nonsmokers were more likely than smokers to develop possible GTS symptoms. In other words, nonsmokers may be especially vulnerable to GTS (26), (30). The reason for this is presumably because smokers are generally more tolerant to nicotine, and therefore they are less likely to have symptoms that can be caused by additional nicotine exposure (100). Similarly, a number of previous studies (5), (25), (26), (30), (101), have revealed that use of tobacco products (smoking or smokeless) appears to decrease absorption of nicotine and that the dermal absorption variable "smoking tobacco" has a significant inverse relationship with GTS incidence (27). On the other hand, the present study found that there was only one tobacco farmer who also smoked cigarettes who had subjective health symptoms which met the definition of GTS.

This study aimed to test the hypothesis that a positive association existed between salivary cotinine level and GTS among Thai traditional tobacco farmers and to describe the salivary cotinine levels of tobacco farmers involved in dried tobacco production. The study also aimed to conduct a follow-up study to determine whether or not Thai traditional tobacco farmers absorbed nicotine from the tobacco leaves they were exposed to. Gas chromatography-nitrogen phosphorous detection (GC) is a valid, reliable, and commonly used quantitative method to measure cotinine in human urine or saliva (102). However, GC is a time-consuming and relatively expensive method. An alternative method that was chosen in the present study was the NicAlert™ saliva strips test (NCTS) because the test can detect as little as 10 ng/mL cotinine. Furthermore, it requires minimal training to use reliably, can be used anywhere, and provides result within approximately 30 minutes only. In general, providing a urine sample is often unacceptable to people and it is rather difficult to arrange in some settings, but collecting saliva specimen is likely to be more acceptable (103). The diagnosis accuracy of NCTS when used with saliva was 99% sensitivity and 96% specificity (104). NCTS detects exposure to nicotine from all sources (e.g., nicotine replacement therapy, chewing tobacco, smoking a cigar, and being secondhand smokers (SHS), not just nicotine exposure from cigarettes (105).

In this study, it was found that NCTS could detect cotinine levels in tobacco farmers and non-tobacco farmers, and the correlation between salivary cotinine levels and nicotine exposure among tobacco farmers was different from that among non-farmers with statistical significance. In addition, NCTS may be a physical tool to witness nicotine exposure among non-farmers who did not work with tobacco, smoke, live with smokers, or who were not secondhand smokers (SHS). Our analysis showed GTS prevalence in each time of the test, and the test results could be used to describe the internal dose of nicotine, as estimated by salivary cotinine levels that reflected the relationship between the process of dried Thai traditional tobacco production and GTS. Such findings were consistent with the findings of a study undertaken by Kongtip et al. (2009) who found that the nicotine dust exposure from dermal route may promote the absorption of nicotine from dust more than direct inhalation because of excessive moisture from sweat in the summer. Also, moisture promoted GTS among the tobacco harvesters (5). Additionally, workers who worked for all of the day and every day may be exposed to nicotine dust through both inhalation and dermal contact for prolonged periods of time and developed some symptoms (19) which seemed to be related to GTS. Moreover, a correlation between use of personal protective equipment (PPE) during the dried tobacco production process and salivary cotinine levels was also found. In fact, lack of use of PPE is considered one of the risk factors of GTS. Similarly, Arcury et al. (2003) investigated the internal dose of nicotine, as estimated by salivary cotinine, and reported on the relationship between work behaviors and GTS (11). The detection of nicotine poisoning from dried Thai traditional tobacco production via inhalation or dermal absorption must be carefully considered to better determine the specific effects of different routes of exposure. Besides this, the findings of this study indicated that farmers who had the nausea symptom consistent with GTS did not appear to have any correlation with salivary cotinine levels. This may be explained that nausea may not be caused by a low level of concentration of salivary cotinine levels. Also, the correlation between the symptom of dizziness and cotinine levels was found only in first NCTS test. One plausible explanation is that dizziness may subside once the farmers had more tolerance with nicotine poisoning (100). Finally, tobacco farmers who wore a face mask as a necessary protective tool to prevent themselves from inhaling a pungent odor of dried tobacco could reduce inhalation exposure as well.

GTS prevention should be based on methods to reduce nicotine absorption. In this study, a high correlation between use of personal protective equipment and salivary cotinine levels was found, particularly when tobacco farmers wore a long-sleeved shirt, gloves, and a face mask. In order to be accepted as providing sufficient protection, the suit and gloves should be lightweight and comfortable enough for tobacco farmers who have to work in a hot climate (5), (106). However, it is noteworthy that in previous studies, it was found that the farmers believed in the protective effects of tobacco use and work experience (11). Apparently, Thai traditional tobacco farmers may not ever believe that smoking is the most effective prevention method of GTS, as only 10% of the farmers in the present study smoked cigarettes and had been smoking for a long time all through their long professional life of tobacco cultivation.

CHAPTER VI CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions and Recommendations

Dermal exposure is a major risk for GTS. The results of the present study represent the first investigation studying the prevalence of GTS among Thai traditional tobacco farmers in Nan Province, Thailand. The findings has indicated that working in wet conditions doing activities such as watering and working with tobacco plants is related to skin integrity like a rash on skin, which may increase absorption of nicotine from tobacco plants. Moreover, insufficient use of PPE may increase health symptoms related to GTS. For these reasons, health education programs that discuss health risks exposure reduction are recommended.

The study results revealed that the process of Thai traditional tobacco cultivation which involves contact with nicotine and pesticides through dermal exposure is a major risk of GTS. The present study is considered the first of its kind to find out the association between symptoms of GTS and symptoms of the levels of AChE and PChE that occur after exposure to pesticides among Thai traditional tobacco farmers in Nan Province, Thailand. The results have indicated that the safe levels of AChE and PChE are associated with symptoms of low dose pesticide exposure and could possibly explain an association between the safe level of AChE and PChE and nicotine poisoning. Based on such results, it is recommended that a health education program is needed to disseminate knowledge and information regarding health risks of exposure to nicotine and pesticides to raise awareness of Thai traditional tobacco farmers. Also, further studies should be conducted to shed more light on long-term effects of exposure to both nicotine and pesticides among Thai traditional tobacco farmers.

The findings of this study indicated that a number of famers always used proper PPE in order to protect themselves from symptoms caused by nicotine exposure during their work. However, it is noteworthy that some farmers may have misunderstood that some PPE such as rubber latex gloves may give them full protection from GTS when, in fact, it is possible to lead to GTS due to sweat or moisture of sweat during the time when the gloves are used. A strong association between a good practice of changing wet clothes after work and GTS is the most remarkable finding of this study. Moreover, the findings help confirm that less use of personal protective equipment may increase adverse health symptoms related to GTS. Finally, a health education program regarding health risk exposure to increase awareness of farmers is recommended, and long-term effect of exposure should be investigated in further studies.

This analysis has indicated that GTS continues to be a common occupational disease among Thai traditional tobacco farmers who cultivate and produce dried Thai traditional tobacco. The present study is the first analysis to find out the correlation between salivary cotinine levels as measured with the strip test called NCTS and dried tobacco production, use of personal protective equipment, and occurrences of GTS. The NCTS is considered both valid and reliable compared to the GC saliva test (105). In addition, to measure cotinine levels in saliva with the NCTS may be more preferable for tests in the field with a large population because the NCTS is able to detect exposure to nicotine from all sources (e.g., nicotine replacement therapy, chewing tobacco, smoking cigar, and being secondhand smokers (SHS), not just from cigarettes. This study has demonstrated the usefulness of using salivary cotinine levels measured with the NCTS as it clearly reflected nicotine exposure among farmers who worked in dried tobacco production. Salivary cotinine levels were also found to be significantly correlated with the prevalence of GTS among tobacco farmers in any time of tests across the crop season in this study, even though this process was different from that used in previous studies when GTS and salivary cotinine levels were correlated in workers who worked in a wet condition that allowed nicotine to penetrate through the skin of their hands. Finally, although the short-term effects of such nicotine exposure may be symptoms of nicotine poisoning which are referred to as GTS and which were the focuses of the present study, the long-term effects of such exposure should be further investigated. A health education program is also recommended to disseminate knowledge and promote understanding of health risks caused by nicotine exposure so as to increase awareness of tobacco farmers and ensure their health as well.

From this finding can explain with diagram that making for more understanding and summarized findings with GTS on Thai traditional tobacco farmers; Based on bio-behavioral model for disease causation, GTS could be integrated such as pesticide exposure that mean AChE and PChE depression on the farmers could be contribute to GTS. The findings has indicated that working in wet conditions doing activities such as watering and working with tobacco plants is related to skin integrity like a rash on skin, which may increase absorption of nicotine from tobacco plants. This study has demonstrated the usefulness of using salivary cotinine levels measured with the NCTS as it clearly reflected nicotine exposure among farmers who worked in dried tobacco production. Salivary cotinine levels were also found to be significantly correlated with the prevalence of GTS among tobacco farmers in any time of tests across the crop season in this study. However, the people who did not working with tobacco plant can be detected by NCTS and this study was found cotinine levels in the body that could be explain the exposure of nicotine via inhalation were possible to find out in this areas.

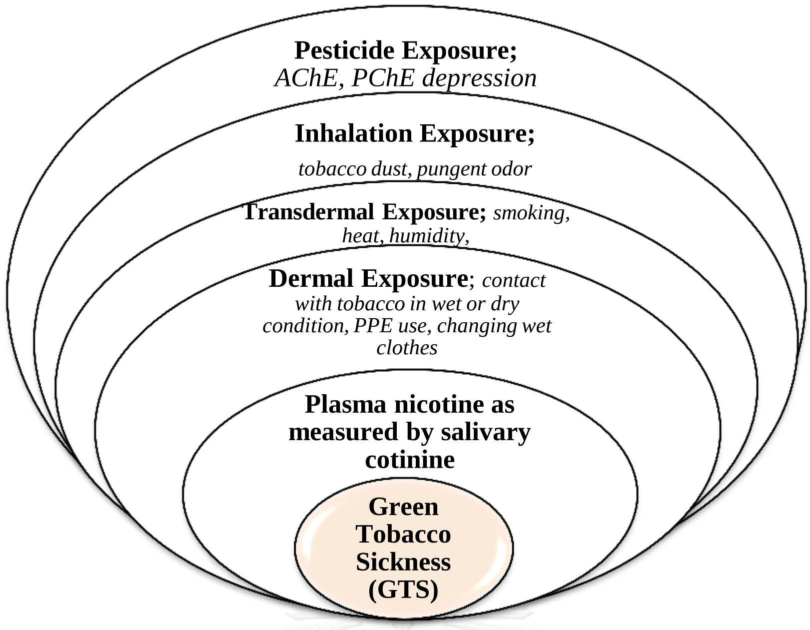


Figure 3: Bio-behavioral model of green tobacco sickness (GTS) causation for Thai traditional tobacco farmers.(Adapted from Arcury et al., 2001; Quandt et al., 2000)

Finally, the health education program with health risk exposure for increase awareness of farmers is recommended and long- term effect of exposure should be investigated.

6.2 Limitation

6.2.1 GTS and prevalence

The limitations of this study should be noted. It is difficult to estimate the true prevalence of GTS based on different definitions of GTS. .As in many GTS investigations earlier conducted; there are no established criteria for the diagnosis of GTS. GTS should be verified with a biomarker of GTS. In this study, the definition of GTS refers to symptoms that occur after expose to tobacco leaves within two to three days with typical symptoms including headache, nausea, vomiting, and dizziness, as well as other less common but possible symptoms of blurred vision, weakness, runny eyes, increased salivation, and increased perspiration.(2), (20), (33)

6.2.2 GTS and Pesticide exposure levels

This study was a cross-sectional research study, and it is acknowledged that uncertainty in time relationship could cloud interpretation of this type of study. Moreover, the validity of data collection depended partly on characteristics of both the interviewer and study participants. In fact, characteristics of the participants including their ability to recall the data and their willingness to answer the questions asked by the interviewer may have an influence on the validity of the study results. Therefore, during data collection, the researchers intended to overcome such a limitation by providing clear explanation to the farmers before data collection commenced.

6.2.3 GTS and Personal protective behaviors

As in many studies of GTS, the definitions of GTS followed those given in previous studies as there are no established criteria for the diagnosis of GTS. Thus, a direct assessment of GTS, or biomarkers of GTS, should be identified and verified. In addition, these study analyses lack a direct measurement of GTS, or a biomarker of GTS. The measurements used in this study have been used in previous studies, with correlation to cotinine levels (10), (11). A validated questionnaire is also needed, and the combined symptoms of headache, dizziness, nausea, and vomiting occurred only among those who had worked in Thai traditional tobacco farm (92).

6.2.4 GTS and Salivary cotinine levels

Some limitations of this study should be noted. First and foremost, individual variability in terms of metabolism and clearance of cotinine and nicotine could have had an effect on the levels of cotinine detected in their saliva. Secondly, it was also possible that the occurrence of GTS may have been overestimated because the symptoms of GTS are nonspecific, and some individuals with other subjective health symptoms such as heat stress or dehydration could have been mistakenly included. Finally, the numbers reported in the individual studies generally depend on the case definitions applied and health beliefs include the awareness of stakeholders that the condition GTS exists at all.

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APPENDIX

Questionnaire

Ethical approval document

BIQ 1 (Individual questionnaire)

BIQ2 (Follow-up individual questionnaire)

AF 01-12

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย
อาคารสถาบัน 2 ชั้น 4 ซอยจุฬาลงกรณ์ 62 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330
โทรศัพท์: 0-2218-8147 โทรสาร: 0-2218-8147 E-mail: eccu@chula.ac.th

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COA No. 170/2555

ใบรับรองโครงการวิจัย

โครงการวิจัยที่ 121.2/55 : กลุ่มอาการป่วยจากการสัมผัสใบยาสูบของเกษตรกรผู้ปลูกยาสูบพันธุ์พื้นเมืองในจังหวัดน่าน ประเทศไทย

ผู้วิจัยหลัก : นายธนูศิลป์ สลีอ่อน

หน่วยงาน : วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย ได้พิจารณา โดยใช้หลัก ของ The International Conference on Harmonization – Good Clinical Practice (ICH-GCP) อนุมัติให้ดำเนินการศึกษาวิจัยเรื่องดังกล่าวได้

ลงนาม..	**ลงนาม**..
(รองศาสตราจารย์ นายแพทย์ปรีดา ทัศนประดิษฐ)	(ผู้ช่วยศาสตราจารย์ ดร.นันทรี ชัยชนะวงศาโรจน์)
ประธาน	กรรมการและเลขานุการ

วันที่รับรอง : 2 ธันวาคม 2555 **วันหมดอายุ** : 1 ธันวาคม 2556

เอกสารที่คณะกรรมการรับรอง

1) โครงการวิจัย
2) ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัยและใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย
3) ผู้วิจัย
4) แบบสอบถาม

เลขที่โครงการวิจัย 121.2/55
วันที่รับรอง -2 ธ.ค. 2555
วันหมดอายุ -1 ธ.ค. 2556

<u>เงื่อนไข</u>

1. ข้าพเจ้ารับทราบว่าเป็นการผิดจริยธรรม หากดำเนินการเก็บข้อมูลการวิจัยก่อนได้รับการอนุมัติจากคณะกรรมการพิจารณาจริยธรรมการวิจัยฯ
2. หากใบรับรองโครงการวิจัยหมดอายุ การดำเนินการวิจัยต้องยุติ เมื่อต้องการต่ออายุต้องขออนุมัติใหม่ล่วงหน้าไม่ต่ำกว่า 1 เดือน พร้อมส่งรายงานความก้าวหน้าการวิจัย
3. ต้องดำเนินการวิจัยตามที่ระบุไว้ในโครงการวิจัยอย่างเคร่งครัด
4. ใช้เอกสารข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย ใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย และเอกสารเชิญเข้าร่วมวิจัย (ถ้ามี) ***เฉพาะที่ประทับตราคณะกรรมการเท่านั้น***
5. หากเกิดเหตุการณ์ไม่พึงประสงค์ร้ายแรงในสถานที่เก็บข้อมูลที่ขออนุมัติจากคณะกรรมการ ต้องรายงานคณะกรรมการภายใน 5 วันทำการ
6. หากมีการเปลี่ยนแปลงการดำเนินการวิจัย ให้ส่งคณะกรรมการพิจารณารับรองก่อนดำเนินการ
7. โครงการวิจัยไม่เกิน 1 ปี ส่งแบบรายงานสิ้นสุดโครงการวิจัย (AF 03-12) และบทคัดย่อผลการวิจัยภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น สำหรับโครงการวิจัยที่เป็นวิทยานิพนธ์ให้ส่งบทคัดย่อผลการวิจัย ภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น

ID. Code…………

QUESTIONNAIRE: BIQ 1

(Individual questionnaire)

(English Version)

Green Tobacco Sickness (GTS) in Thai Traditional Tobacco Farmers Related to Their Occupational Exposure in Nan Province, Thailand

Explanation

This questionnaire is prepared for Green Tobacco Sickness (GTS): Occupational Exposure, Biomarker and Subjective Health Symptoms among Thai Traditional Tobacco Farmers in Nan Province, Thailand consists of four parts 5 parts

Part 1 General data and Socio-demographic information

Part 2 Dermal exposure to Nicotine

Part 3 Transdermal absorption

Part 4 Self-reported symptoms

Part 5 Salivary cotinine Test record and AChE/PChE

Please answer this Information to straight, we wish receive an actual information for this study and bring it to planning for support a suitable prevention of health adverse from nicotine poisoning in the future. Including apply for benefit in support knowledge in people to prevent themselves from nicotine poisoning in Thai traditional tobacco farmer other area.

Name…………………………..surname………………………..

ID													

House number…………………Village Name………………Village NO…………

…………Sub-District,…………………District, Nan Province, Thailand.

Name…………………..surname…………D/M/Y…………..Interviewer

Your answers will not be released to anyone and will remain anonymous. Presentation a research result in overall image not refers into an individual in report.

Thank you for your assistance.

BIQ 1 (Individual questionnaire)

Part 1 General data and Socio-demographic information

Interviewer: Place an X in the box of the selected answer(s).

General data and Socio-demographic information	For Researcher
1. Gender 1 ( ) Male 2 ( ) Female	
2. Role status in family 1 ( ) Head of family 2 ( ) Housewife 3 ( ) Member 4 ( ) Resident 5 ( ) Other.......................	
3. Age……………years 1 ( ) < 20 years 2 ( ) 20-29 years 3 ( ) 30-39 years 4 ( )40-49 years 5 ( ) 50-59 years 6 ( ) ≥ 60 years	
4.Marital Status 1 ( ) Single 2 ( ) Couple 3 ( )Widowed, divorce and Separate	
5. Education Level 1 ( ) Primary 2 ( ) Lower secondary school 3 ( ) High school 4 ( ) Certificate level 5 ( )University level or upper 6( ) no study	
6. Occupation 1 ( ) Agriculturist 2 ( ) Merchant 3 ( ) Employee labor 4 ( ) Government employee 5 ( ) Student 6 ( )Other.............	
7. Land owner 1 ( )Yes 2 ( ) No	
8. Monthly income…………………Baht	
9. Pesticide applied within previous 2-3 days 1 ( ) Yes 2 ( ) No	
10. Health problem 1 ( ) Yes 2 ( ) No	
11. Chronic Disease 1( ) Hypertension 2( ) Diabetes 3( ) Heart 4( ) Others ………………….	

Part 2 Dermal exposure to Nicotine

Interviewer: Place an / in the box of the selected answer(s).

2. 1 High…………….cms. Weigh…………………Kg BMI……………

2. 2Worked in Thai Traditional Tobacco farming (year)

1. ( ) 1-3 years 2.( ) 4-10 years 3.( ) 11-20 years

4.( ) >20 years

2.3. Contact with tobacco farming

When you worked with tobacco, you…..	Answer		For Researcher
	Yes	No	
2.3.1. wear long sleeve shirt			
2.3.2 wear long pants			
2.3.3 wear rain suit			
2.3.4 wear plastic protect			
2.3.5 change out of wet clothes after worked			
2.3.6 wear glove			
2.3.7 wear boots			

Part 3 Transdermal absorption

Interviewer: Place an / in the box of the selected answer(s).

3.1 Tobacco Use

1 ( ) smoke 2( ) Non smoke 3( ) passive smoking

4( ) Smoked…….years

3.2 **Smoking; Cigarette/Rolling cigarette** 1( ) < 1 pack 2( ) ≥ 1pack

3.3 **Skin integrity** 1( ) Rash 2( ) Cuts 3( ) abrasion 4( ) Normal

3.4 **Alcohol consumption** 1( ) Yes 2 ( ) No

3.5 **Work in wet clothes** 1( ) Yes 2 ( ) No

3.6 **Humidity**………..%

3.7 **Heat** (average mean temperature)……………..'C

Part 4 Self-reported symptoms after contact with Thai Traditional Tobacco

Interviewer: Place an / in the box of the selected answer(s).

After contact with Thai Traditional Tobacco within 2-3 days, Have you ever been with these symptoms?

Symptoms	Within2- 3 Days before		Today		For Researcher
	Yes	No	Yes	No	
1. Headache					
2. Nausea					
3. Vomiting					
4. weakness					
5.Dizziness					
6. Runny eyes					
7. Blurred vision					
8. Increased perspiration					
9. Increased salivation					

Part 5 Salivary Cotinine levels Test by NicAlert™ Saliva test

Interviewer: Please check / in the blank box of the result of the test.

Test Date…………./………………./……………

Level	**Cotinine Concentration (ng/mL)**	**Result**	**For Researcher**
0	0-10		
1	10-30		
2	30-100		
3	100-200		
4	200-500		
5	500-1000		
6	>1000		

Acetylcholinesterase …………..U/ml. ; Plasma cholinesterase ……………….U/ml.

ID. Code…………

QUESTIONNAIRE: BIQ2
(Follow-up individual questionnaire)

(English Version)

Green Tobacco Sickness (GTS) in Thai Traditional Tobacco Farmers Related to Their Occupational Exposure in Nan Province, Thailand

Explanation

This questionnaire is prepared for Green Tobacco Sickness (GTS) in Thai Traditional Tobacco Farmers Relate to Their Occupational Exposure in Nan Province, Thailand: consists of four parts 4 parts

Part 1 Dermal exposure to Nicotine

Part 2 Transdermal absorption

Part 3 Self-reported symptoms

Part 4 Salivary cotinine Test record

Please answer this Information to straight, we wish receive an actual information for this study and bring it to planning for support a suitable prevention of health adverse from nicotine poisoning in the future. Including apply for benefit in support knowledge in people to prevent themselves from nicotine poisoning in Thai traditional tobacco farmer other area.

Name…………………………..surname………………………..

ID													

House number…………………Village Name………………Village NO……….

…………….Sub-District,………………………. District, Nan Province, Thailand.

Name…………………..surname…………D/M/Y…………..Interviewer

Your answers will not be released to anyone and will remain anonymous. Presentation a research result in overall image not refers into an individual in report.

Thank you for your assistance.

BIQ 2 (Follow-up individual questionnaire)

Part 1 Dermal exposure to Nicotine (Within previous 2-3 days)

Interviewer: Place an X in the box of the selected answer(s).

1. Contact with tobacco farming

When you worked with tobacco, you…..	Answer		For Researcher
	Yes	No	
1.1 wear long sleeve shirt			
1.2 wear long pants			
1.3 wear rain suit			
1.4 wear plastic protect			
1.5 change out of wet clothes after worked			
1.6 wear glove			
1.7 wear boots			

Part 2 Transdermal absorption (Within previous 2-3 day)

Interviewer: Place an / in the box of the selected answer(s).

3.1 Tobacco Use

1 ( ) smoke 2( ) Non smoke 3( ) passive smoking

4( ) Smoked…….years

3.2 **Smoking; Cigarette/Rolling cigarette** 1( ) < 1 pack 2( ) ≥ 1pack

3.3 **Skin integrity** 1( ) Rash 2( ) Cuts 3( ) abrasion 4( ) Normal

3.4 A**lcohol consumption** 1( ) Yes 2 ( ) No

3.5 **Work in wet clothes** 1( ) Yes 2 ( ) No

3.6 **Humidity**………..

3.7 **Heat** (average mean temperature)……………..'C

Part 3 Self-reported symptoms after contact with Thai Traditional Tobacco

Interviewer: Place an / in the box of the selected answer(s).

After contact with Thai Traditional Tobacco within 2-3 days, have you ever been with these symptoms?

Symptoms	Within2- 3 Days before		Today		For Researcher
	Yes	No	Yes	No	
1. Headache					
2. Nausea					
3. Vomiting					
4. weakness					
5.Dizziness					
6. Runny eyes					
7. Blurred vision					
8. Increased perspiration					
9. Increased salivation					

Others symptoms…………………………………………………………………………...

………………………………………………………………………………………………

Part 4 Salivary Cotinine levels Test by NicAlert™ saliva strips

Name…………………………..surname………………………..

ID													

House number…………………Village Name………………Village NO

…………...Sub-District,……………………………District, Nan Province, Thailand.

Name…………………..Surname…………D/M/Y…………..Interviewer

Items	Test 1 Date………	Test 2 Date………… .	Test 3 Date………… .	For Researcher	
Salivary Cotinine levels					

Items	Test 4 Date………	Test 5 Date…………	Test 6 Date………… .	For Researcher	
Salivary Cotinine levels					

Items	Test 7 Date…………
Salivary Cotinine levels	

เลขที่แบบสอบถาม [ ][ ][ ]

แบบสอบถาม

BIQ 1 ข้อมูลพื้นฐานรายบุคคล

กลุ่มอาการกรีนโทแบคโค่ซิกเนส เนื่องจากการประกอบอาชีพในเกษตรกรผู้ผลิต ใบยาสูบพันธุ์พื้นเมืองในจังหวัดน่าน ประเทศไทย

คำชี้แจง

แบบสอบถามชุดนี้จัดทำขึ้นเพื่อศึกษาการเกิด กลุ่มอาการกรีนโทแบคโค่ซิกเนส อาชีพเหตุสัมผัส ดัชนีชีวภาพและกลุ่มอาการสุขภาพในเกษตรกรผู้ผลิตใบยาสูบพันธุ์พื้นเมืองในจังหวัดน่าน ประเทศไทย โดยแบบสอบถามมีทั้งหมด 4 หน้า แบ่งออกเป็น

ส่วนที่ 1	ข้อมูลทั่วไปและลักษณะทางประชากร	จำนวน 11 ข้อ
ส่วนที่ 2	แบบสอบถามการสัมผัสนิโคตินทางผิวหนัง	จำนวน 9 ข้อ
ส่วนที่ 3	แบบสอบถามการดูดซึมทางผิวหนัง	จำนวน 7 ข้อ
ส่วนที่ 4	แบบสอบถามเกี่ยวกับอาการที่พบ	จำนวน 9 ข้อ
ส่วนที่ 5	แบบบันทึกผลการตรวจระดับโคตินินในน้ำลายและ AChE/PChE	

โปรดตอบแบบสอบถามนี้ให้ตรงกับความเป็นจริง เพื่อที่จะนำข้อมูลที่ได้ ไปใช้เป็นแนวทาง ในการศึกษาและหาข้อเสนอแนะ และใช้ในการวางแผนส่งเสริมความรู้ และการปฏิบัติตน ที่ถูกต้องเหมาะสม เพื่อป้องกันไม่ให้เกิดการอาการผิดปกติทางสุขภาพเนื่องจากสัมผัสนิโคตินทางผิวหนัง จากกิจกรรมที่เกี่ยวข้องกับใบยาสูบพันธุ์พื้นเมือง รวมทั้งปรับใช้ให้เกิดประโยชน์ แก่ประชาชน ในการที่จะส่งเสริมให้ความรู้ในการป้องกันอาการไม่พึงประสงค์ แก่ประชาชนในพื้นที่ อื่นๆ ที่ประกอบอาชีพปลูกยาสูบพันธุ์พื้นเมือง ต่อไป

ชื่อ ....สกุล..ผู้ให้สัมภาษณ์

หมายเลขประจำตัวบัตรประชาชน

บ้านเลขที่............ บ้าน............หมู่ที่...............ตำบล........................ อำเภอ.................... จังหวัด าน

ชื่อ....................................สกุล.........................วัน/เดือน/ปี..............ผู้สัมภาษณ์

ขอขอบพระคุณทุกท่านในการตอบแบบสอบถาม

วันที่…….เดือน………..…….พ.ศ. ………

ส่วนที่ 1 ข้อมูลทั่วไป

คำชี้แจง : โปรดใส่เครื่องหมาย X ลงใน ( ) หรือกรอกข้อความลงในช่องว่าง ที่ตรงกับความเป็นจริง

เลขที่แบบสอบถาม [ ][ ][ ]

ข้อมูลทั่วไป	**สำหรับผู้วิจัย**
1. เพศ 1 ( ) ชาย 2 ( ) หญิง	
2. บทบาทสถานะในครอบครัว 1 ( ) หัวหน้าครอบครัว 2 ( ) แม่บ้าน 3 ( ) สมาชิก 4 ผู้อาศัย ( ) 5 ( ) อื่นๆ…………………….	
3. อายุ…………….ปี (เต็ม) 1 ( ) น้อยกว่า 20 ปี 2 ( ) 20-29 ปี 3 ( ) 30-39 ปี 4 ( )40-49 ปี 5 ( ) 50-59 ปี 6 ( ) ≥ 60 ปี	
4. สถานภาพ 1 ( ) โสด 2 ( ) คู่ 3 ( ) ม่าย หย่า แยก	
5. การศึกษาสูงสุด 1 ( ) ประถมศึกษา 2 ( ) มัธยมศึกษาต้น 3 ( ) มัธยมศึกษาปลาย 4 ( ) อนุปริญญา/ปวส. 5 ( ) ปริญญาตรีขึ้นไป 6 ( ) ไม่ได้เรียน	
6. อาชีพหลัก 1 ( ) เกษตรกรรม 2 ( ) ค้าขาย 3 ( ) รับจ้าง 4 ( ) รับราชการ / รัฐวิสาหกิจ 5 ( ) นักเรียน / นักศึกษา 6 .... อื่นๆ ( )	

ข้อมูลทั่วไป	**สำหรับผู้วิจัย**
7. ท่านเป็นเจ้าของไร่ยาสูบพันธุ์พื้นเมืองหรือไม่ 1 ใช่ ( )2 ( ) ไม่ใช่	
8. รายได้เฉลี่ยของท่าน บาท ต่อเดือน..................................	
9. การใช้ยาฆ่าแมลงใน 2-3 วันที่ผ่านมา 1 ( ) ใช้2 ( ) ไม่ใช้	
10. ท่านมีปัญหาด้านสุขภาพหรือไม่ 1 ( )มี 2( ) ไม่มี	
11. ท่านมีโรคประจำตัวหรือไม่ 1( ) ความดันโลหิตสูง 2( ) เบาหวาน 3( ) หัวใจ 4( ) อื่นๆ ระบุ...............	

ส่วนที่ 2 แบบสอบถามเกี่ยวกับการสัมผัสนิโคตินทางผิวหนังจากใบยาสูบ

คำชี้แจง : โปรดใส่เครื่องหมาย X ลงใน ( ) หรือกรอกข้อความลงในช่องว่าง ที่ตรงกับความเป็นจริง

2.1 ส่วนสูง......................เซนติเมตร น้ำหนัก.........................กิโลกรัม ดัชนีมวลกาย...................

2.2 ประสบการณ์ในการปลูกยาสูบพันธุ์พื้นเมือง

1. ( ) 1-3 ปี 2.( ) 4-10 ปี 3.( ) 11-20 ปี 4.( ) มากกว่า 20 ปี

2.3 การสัมผัสทางผิวหนังกับใบยาสูบ

เมื่อท่านทำงานที่เกี่ยวข้องและสัมผัสกับใบยาสูบพันธุ์พื้นเมือง	การปฏิบัติ		สำหรับนักวิจัย
	ใช่	ไม่ใช่	
2.3.1. สวมเสื้อแขนยาว			
2.3.2 สวมกางเกงขายาว			
2.3.3 สวมเสื้อกันฝน			
2.3.4 สวมใส่พลาสติกกันเปื้อน			
2.3.5 เปลี่ยนชุดที่เปียกหลังเลิกงาน			
2.3.6 สวมถุงมือ			
2.3.7 สวมรองเท้าบูท			

ส่วนที่ 3 แบบสอบถามเกี่ยวกับการดูดซึมทางผิวหนังที่เกี่ยวข้อง

คำชี้แจง: โปรดใส่เครื่องหมาย X ลงใน ( ) หรือกรอกข้อความลงในช่องว่าง ที่ตรงกับความเป็นจริง

การดูดซึมทางผิวหนัง	**สำหรับผู้วิจัย**
1. ท่านสูบบุหรี่หรือไม่ 1 ( ) สูบ 2 ( ) ไม่สูบ 3 ( ) อาศัยอยู่กับผู้ที่สูบบุหรี่ 4 ( ) เลิกสูบปี......	
2. สูบบุหรี่ก้นกรอง/ยาเส้น 1 ( ) น้อยกว่า 1ซอง 2 ( ) มากกว่าหรือเท่ากับ1 ซอง	
3. ความสมบูรณ์ของผิวหนังท่าน 1 ( ) มีผื่นคัน 2 ( ) มีแผล 3( ) รอยถลอก 4( ) ปกติ	
4. การดื่มเครื่องดื่มแอลกอฮอล์ 1 ( ) ดื่ม 2 ( ) ไม่ดื่ม	
5. ทำงานในขณะที่เสื้อผ้าเปียก 1( ) ใช่ 2( ) ไม่ใช่	
6. ความชื้นสัมพัทธ์ ร้อยละ.......................	
7. อุณหภูมิเฉลี่ยองศาเซลเซียส......................................	

ส่วนที่ 4 แบบสอบถามเกี่ยวกับอาการที่เกิดขึ้นภายหลังเก็บใบยาสูบในระยะ 2-3 วันและวันที่สัมภาษณ์

คำชี้แจง : ให้ทำเครื่องหมาย / ลงในช่อง □

กลุ่มอาการ	ภายใน **2-3**วันก่อนหน้านี้		วันนี้		สำหรับนักวิจัย
	มี	ไม่มี	มี	ไม่มี	
1. ปวดศรีษะ					
2. คลื่นไส้					
3. อาเจียน					
4.อ่อนเพลีย					
5.วิงเวียนศรีษะ					
6. น้ำตาไหล					
7.เห็นภาพไม่ชัด					
8. เหงื่อออกมาก					
9. มีน้ำลายมากขึ้น					

ส่วนที่ 5 แบบบันทึกระดับความเข้มข้นของโคตินินที่พบในน้ำลาย

คำชี้แจง : ให้ทำเครื่องหมาย/ลงในช่อง □

ระดับ	ความเข้มข้นของโคตินิน (ng/mL)	ผลการทดสอบ	**สำหรับนักวิจัย**
0	0-10		
1	10-30		
2	30-100		
3	100-200		
4	200-500		
5	500-1000		
6	>1000		

ระดับ Acetylcholinesterase ....U/ml. ; Plasma cholinesterase ....U/ml.

เลขที่แบบสอบถาม [ ][ ][ ]

แบบสอบถาม

BIQ 2 การติดตามและสัมภาษณ์รายบุคคล

กลุ่มอาการกรีนโทแบคโค่ซิกเนส เนื่องจากการประกอบอาชีพในเกษตรกรผู้ผลิต ใบยาสูบพันธุ์พื้นเมืองในจังหวัดน่าน ประเทศไทย

คำชี้แจง

แบบสอบถามชุดนี้จัดทำขึ้นเพื่อศึกษาการเกิด กลุ่มอาการกรีนโทแบคโค่ซิกเนส อาชีพเหตุสัมผัส ดัชนีชีวภาพและกลุ่มอาการสุขภาพในเกษตรกรผู้ผลิตใบยาสูบพันธุ์พื้นเมืองในจังหวัดน่าน ประเทศไทย โดยแบบสอบถามมีทั้งหมด 4 หน้า แบ่งออกเป็น

ส่วนที่ 1	แบบสอบถามการสัมผัสนิโคตินทางผิวหนัง	จำนวน 7 ข้อ
ส่วนที่ 2	แบบสอบถามการดูดซึมทางผิวหนัง	จำนวน 7 ข้อ
ส่วนที่ 3	แบบสอบถามเกี่ยวกับอาการที่พบ	จำนวน 9 ข้อ
ส่วนที่ 4	แบบบันทึกผลการตรวจระดับโคตินินในน้ำลาย	

โปรดตอบแบบสอบถามนี้ให้ตรงกับความเป็นจริง เพื่อที่จะนำข้อมูลที่ได้ ไปใช้เป็นแนวทาง ในการศึกษาและหาข้อเสนอแนะ และใช้ในการวางแผนส่งเสริมความรู้ และการปฏิบัติตน ที่ถูกต้องเหมาะสม เพื่อป้องกันไม่ให้เกิดการอาการผิดปกติทางสุขภาพเนื่องจากสัมผัสนิโคตินทางผิวหนัง จากกิจกรรมที่เกี่ยวข้องกับใบยาสูบพันธุ์พื้นเมือง รวมทั้งปรับใช้ให้เกิดประโยชน์ แก่ประชาชน ในการที่จะส่งเสริมให้ความรู้ในการป้องกันอาการไม่พึงประสงค์ แก่ประชาชนในพื้นที่ อื่นๆ ที่ประกอบอาชีพ ปลูกยาสูบพันธุ์พื้นเมือง ต่อไป

ชื่อ ....สกุล...ผู้ให้สัมภาษณ์

หมายเลขประจำตัวบัตรประชาชน

บ้านเลขที่............ บ้าน............หมู่ที่...............ตำบล....................... อำเภอ.................... จังหวัด น่าน

ชื่อ.......................................สกุล...............................วัน/เดือน/ปี..............ผู้สัมภาษณ์

ขอขอบพระคุณทุกท่านในการตอบแบบสอบถาม

เลขที่แบบสอบถาม [ ][ ][ ]

วันที่…….เดือน………..…….พ.ศ. ………

ส่วนที่ 1 แบบติดตามและสอบถามเกี่ยวกับการสัมผัสนิโคตินทางผิวหนังจากใบยาสูบ ภายใน6 วัน

คำชี้แจง : โปรดใส่เครื่องหมาย X ลงใน ( ) หรือกรอกข้อความลงในช่องว่าง ที่ตรงกับความเป็นจริง

เมื่อท่านทำงานที่เกี่ยวข้องและสัมผัสกับ ใบยาสูบพันธุ์พื้นเมือง	การปฏิบัติ		สำหรับนักวิจัย
	ใช่	ไม่ใช่	
1.1 สวมเสื้อแขนยาว			
1.2 สวมกางเกงขายาว			
1.3 สวมเสื้อกันฝน			
1.4 สวมใส่พลาสติกกันเปื้อน			
1.5 เปลี่ยนชุดที่เปียกหลังเลิกงาน			
1.6 สวมถุงมือ			
1.7 สวมรองเท้าบูท			

ส่วนที่ 2 แบบสอบถามเกี่ยวกับการดูดซึมทางผิวหนังที่เกี่ยวข้อง ภายใน 2-3 วัน

คำชี้แจง: โปรดใส่เครื่องหมาย X ลงใน ( ) หรือกรอกข้อความลงในช่องว่าง ที่ตรงกับความเป็นจริง

การดูดซึมทางผิวหนัง	**สำหรับผู้วิจัย**
1. ท่านสูบบุหรี่หรือไม่ 1 ( ) สูบ 2 ( ) ไม่สูบ 3 ( ) อาศัยอยู่กับผู้ที่สูบบุหรี่ 4( ) เลิกสูบปี......	

การดูดซึมทางผิวหนัง	**สำหรับผู้วิจัย**
2. สูบบุหรี่ก้นกรอง/ยาเส้น 1 ( ) น้อยกว่า 1ซอง 2 ( ) มากกว่าหรือเท่ากับ1 ซอง	
3. ความสมบูรณ์ของผิวหนังท่าน 1 ( ) มีผื่นคัน 2 ( ) มีแผล 3( ) รอยถลอก 4( ) ปกติ	
4. การดื่มเครื่องดื่มแอลกอฮอล์ 1 ( ) ดื่ม 2 ( ) ไม่ดื่ม	
5. ทำงานในขณะที่เสื้อผ้าเปียก 1( ) ใช่ 2( ) ไม่ใช่	
6. ความชื้นสัมพัทธ์ ร้อยละ......................	
7. อุณหภูมิเฉลี่ยองศาเซลเซียส......................................	

ส่วนที่ 3 แบบสอบถามเกี่ยวกับอาการที่เกิดขึ้นภายหลังเก็บใบยาสูบในระยะ 2-3 วันและวันที่สัมภาษณ์

คำชี้แจง : ให้ทำเครื่องหมาย / ลงในช่อง ☐

กลุ่มอาการ	ภายใน **2-3**วันก่อนหน้านี้		วันนี้		สำหรับนักวิจัย
	มี	ไม่มี	มี	ไม่มี	
1. ปวดศีรษะ					
2. คลื่นไส้					
3. อาเจียน					
4.อ่อนเพลีย					
5.วิงเวียนศีรษะ					
6. น้ำตาไหล					
7.เห็นภาพไม่ชัด					
8. เหงื่อออกมาก					
9. มีน้ำลายมากขึ้น					

ส่วนที่ 4 แบบบันทึกระดับความเข้มข้นของโคตินินที่พบในน้ำลาย

คำชี้แจง : ให้ทำเครื่องหมาย/ลงในช่อง ☐

ชื่อ…………………………..สกุล………………………..

ID

บ้านเลขที่…………บ้าน……………หมู่……..ตำบล………………….อำเภอ,………………
จังหวัดน่าน ชื่อผู้สัมภาษณ์…………………..….

………วัน/เดือน/ปี………………………………………….

ดัชนี	**ครั้งที่ 1** **วัน/เดือน/ปี……**	**ครั้งที่ 2** **วัน/เดือน/ปี…….**	**ครั้งที่ 3** **วัน/เดือน/ปี…….**		**สำหรับ นักวิจัย**
ระดับโคตินิน ในน้ำลาย					

ดัชนี	**ครั้งที่ 4** **วัน/เดือน/ปี…**	**ครั้งที่ 5** **วัน/เดือน/ปี…….**	**ครั้งที่ 6** **วัน/เดือน/ปี…….**	**สำหรับ นักวิจัย**	
ระดับโคตินิน ในน้ำลาย					

ดัชนี	ครั้งที่ 7 วัน/เดือน/ปี…….		สำหรับนักวิจัย
ระดับโคตินิน ในน้ำลาย			

VITA

Mr.Thanusin Saleeon was born in Nan Province, Thailand. He received his Diploma of Public health (Public Health) from Sirindhorn Public Health College, Phitsanulok Province, Bachelor of Public Health (Second Class Honours) from Khonkaen University, Bachelor of Public Health from Sukhothaithammathiraj Open University,Master of Public Health (MPH.) from College of Public Health Sciences, Chulalongkorn University. He is a Technical Public Health Officer, Professional Level, and health facilitator contributes to farmers in the rural area and hill tribe people for health promotion and safety working learning. He currently resides in Nan Provincial Public Health Office, Nan Province, the northern of Thailand.