A STUDY OF NEUROBEHAVIOURAL EFFECTS OF LONG-TERM EXPOSURE TO ORGANOPHOSPHATES IN MALE TOBACCO-GROWING FARMERS IN BACHOK, KELANTAN

by

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This Dissertation Is Being Submitted In Partial Fulfilment Of The Requirements For The Degree Of Master of Community Medicine (Occupational Health)

UNIVERSITI SAINS MALAYSIA

November 2001

ACKNOWLEDGEMENTS

I would like to acknowledge the Universiti Sains Malaysia (USM) Short Term Research Grant that made this research project possible. I would like to extend my deepest gratitude to my supervisor, Professor Dr. Rusli Bin Nordin, Professor of Community Medicine/Deputy Dean, School of Dental Sciences, Health Campus USM for making this dissertation possible and for all his guidance, assistance, and cooperation rendered to me throughout my study. Also, to the lecturers and supporting staff at the Department of Community Medicine, School of Medical Sciences, in particular Dr. Syed Hatim Noor and Dr. Mohd Ayub Mohd Sadiq for their statistical advice, and Dr. Kamarul Imran Musa for his technical assistance; Mr. Kamaruddin Hussin, Zahasnam Mohd Noor and Mohd Azwan Abd Rahman for assisting me with the fieldwork. My special thanks goes to the Laboratory Technicians, Chemical Pathology Laboratory, HUSM for their laboratory assistance.

Last but not least, my beloved husband Rosli Abd Ghani, Lt Cdr TLDM, my parents and children, Nur Amalina, Mohamad Amiruddin and Mohamad Amaruddin, who supported and encouraged me throughout my study.

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LIST OF ABBREVIATIONS

ACh	-	Acetylcholine
AChE	-	Acetylcholinesterase
ChE	-	Serum Cholinesterase
EPA	-	Environmental Protection Agency
HUSM	-	Hospital Universiti Sains Malaysia
LTN	-	Lembaga Tembakau Negara
NCTB	-	Neurobehavioural Core Test Battery
NTB	-	National Tobacco Board
OHL	-	Occupational Health Laboratory
OP	-	Organophosphates
PPE	-	Personal Protective Equipment
WHO	-	World Health Organisation

ABSTRAK

Kajian Kesan Neurotingkahlaku Akibat Pendedahan Jangkapanjang Terhadap Organofosfat Di Kalangan Penanam Tembakau Lelaki Di Bachok, Kelantan

Untuk mengkaji pengetahuan mengenai kesan sampingan pestisid ke atas kesihatan, amalan tingkahlaku selamat mengenai penggunaan pestisid dan menyiasat kesan neurotingkahlaku terhadap pekerja yang terdedah secara kronik kepada pestisid organofosfat di kalangan penanam tembakau lelaki di Malaysia, satu kajian irisan lintang komparatif terhadap 45 orang penanam tembakau yang terdedah kepada organofosfat dan 45 orang pekerja HUSM yang tidak terdedah kepada pestisid telah dijalankan dalam bulan Ogos dan September 2000. Soalselidik berpandu mengenai pengetahuan dan amalan dan simptom-simptom subjektif berkaitan dengan penggunaan pestisid di dalam Bahasa Malaysia telah dibentuk dan diprauji. Terdapat 5 bahagian di dalam borang soalselidik; 14 soalan mengenai ciri sosiodemografi dan pekerjaan, 4 soalan mengenai pendedahan kimia lain, 10 soalan mengenai cirri kesihatan umum, 36 soalan mengenai simptom-simptom subjektif dan 20 soalan mengenai amalan selamat berkaitan dengan penggunaan pestisid. Tujuh ujian neurotingkahlaku – profil status mood, min masa reaksi, simbol digit, span digit, deksteriti manual Santa Ana, pengstoran visual Benton dan ujian "pursuit aiming" dijalankan ke atas subjek. Pengukuran kolinesterase serum juga dijalankan.

Keputusan menunjukkan min aktiviti kolinesterase serum penanam tembakau (8924.7±1754.0 U/l) adalah lebih rendah secara signifikan berbanding pekerja HUSM (11421.4±1855.4 U/l), tetapi masih di dalam julat normal (5300 – 12900 U/l).

Peratus penanam tembakau menunjukkan simptom-simptom subjektif yang signifikan seperti terjaga waktu malam, berpeluh berlebihan, kebas lengan, lemah anggota atas, ketar, kebas muka dan kelainan pada otot muka.

Keputusan analisis “covariance” menunjukkan min skor ujian profil status mood “vigour”, simbol digit tepat, span digit ke hadapan dan ke belakang, deksteriti manual Santa Ana tangan tidak dominan, pengstoran visual Benton dan ujian “pursuit aiming” adalah rendah secara signifikan dalam penanam tembakau berbanding pekerja HUSM.

Pengetahuan penanam tembakau mengenai kesan buruk pestisid adalah baik secara amnya kecuali mengenai tanda-tanda awal keracunan di mana terdapat proporsi penanam tembakau yang tidak mengenalpasti loya, muntah dan susah bernafas sebagai tanda-tanda awal keracunan.

Berkenaan dengan amalan mengenai penyimpanan pestisid dan pembuangan bekas pestisid, kebanyakan penanam tembakau menunjukkan amalan yang tidak memuaskan kerana didapati sebahagian besarnya tidak menyediakan tempat khas untuk menyimpan pestisid dan membuang bekas pestisid secara tidak selamat.

Analisa regresi multipel menunjukkan tiada satupun skor ujian neurotingkahlaku 45 orang penanam tembakau yang terdedah mempunyai hubungan yang signifikan dengan aktiviti kolinesterase serum. Bagaimanapun, skor simbol digit tepat, span digit ke belakang, dan ujian “pursuit aiming” adalah berhubung secara signifikan dengan tahap pendidikan. Kami dengan ini membuat kesimpulan bahawa kesan kronik ke atas sistem saraf telah berlaku kepada kumpulan penanam tembakau ini dan kesan-kesan ini adalah berkemungkinan dikaitkan dengan pendedahan kepada pestisid organofosfat secara

berulang-ulang dalam jangkamasa panjang. Langkah-langkah perlu diambil untuk meningkatkan pengetahuan dan amalan penanam tembakau mengenai penggunaan pestisid.

Kata kunci: pestisid organofosfat, pendedahan jangkapanjang, kesan neurotingkahlaku, Bateri Ujian Neurotingkahlaku WHO, penanam tembakau, pengetahuan dan amalan, symptom-simptom subjektif, kolinesterase serum

ABSTRACT

A Study of Neurobehavioural Effects of Long-Term Exposure To Organophosphates In Male Tobacco-Growing Farmers In Bachok, Kelantan

In order to study the knowledge about adverse health effects of pesticides, practice of safe behaviour related to pesticide use and to investigate the neurobehavioural effects in male Malaysian tobacco-growing farmers exposed to OP pesticides, a cross-sectional comparative study of 45 OP-exposed tobacco-growing farmers and 45 non-exposed workers was conducted in August and September 2000. Structured questionnaire on knowledge and practice and subjective symptoms associated with pesticide use in the local Malaysian language was developed and pretested. There were 5 sections in the questionnaires; 14 questions on sociodemographic and occupational characteristics, 4 questions on other chemical exposure, 10 questions on general health characteristics, 36 questions on subjective symptoms checklist, and 20 questions on safety behaviours associated with pesticide use checklist. Seven tests of the NCTB — profile of mood states, simple reaction time, digit symbol, digit span, Santa Ana manual dexterity, Benton visual retention, and pursuit aiming tests — were administered to the subjects. Serum cholinesterase estimation was also undertaken.

Results showed that the mean serum cholinesterase activity in exposed farmers (8924.7±1754.0 U/l) was significantly lower than non-exposed workers (11421.4 ±1855.4 U/l) but within normal range (5300 to 12900 U/l). Proportion of farmers significantly reported more subjective symptoms; waking up at night, excessive sweating, upper limb

numbness, upper limb weakness, tremors, facial numbness, and changes in the facial muscle.

The results of analysis of covariance, in which age, length of education, and duration of employment (covariates) were controlled in 45 exposed farmers and 45 non-exposed, showed that the mean scores on the Profile of Mood States-vigour, digit symbol correct, digit span forward and digit span backward, Santa Ana non-preferred hand, Benton visual retention test and pursuit aiming tests were significantly lower in the exposed farmers than non-exposed workers.

Farmers' knowledge about adverse effects of pesticides is generally good except about early symptoms of poisoning where there is a proportion of farmers who failed to recognise nausea, vomiting, and breathing difficulty as early symptoms of poisoning.

For practice of storage of pesticide and disposal of container, majority of farmers showed unsatisfactory practice as large proportion of farmers failed to provide a special room for pesticide storage and practice safe disposal of container.

The results of stepwise multiple regression analysis revealed that none of the neurobehavioural tests' score for 45 exposed farmers were significantly related to serum cholinesterase. However, scores on the digit symbol correct, digit span backward and pursuit aiming tests were significantly related to length of education. We therefore conclude that chronic effects on the nervous system have occurred in this group of farmers and that these effects are likely to be associated with long-term repeated exposure to organophosphate pesticides. Measures need to be taken to enhance farmers' knowledge and practice of pesticide use.

Key words: organophosphate pesticides, long-term exposure, neurobehavioural effects, WHO Neurobehavioural Core Test Battery, tobacco-growing farmers, knowledge and practice, subjective symptoms, serum cholinesterase

CHAPTER ONE

INTRODUCTION

Rapid development in the Malaysian agricultural sector has resulted in the widespread use of pesticides. Malaysia represents one of the countries with the greatest pesticide consumption apart from Japan, Europe, the United States, China, Brazil, Mexico, Colombia and Argentina (O'Malley 1997). Improper handling and use of pesticides and the lack of knowledge on pesticides safety have led to widespread cases of pesticides poisoning across the country; in 1996, 787 cases of pesticide poisonings were reported in government hospitals throughout Malaysia (Ministry of Health 1997). The proportion of agricultural workers poisoned per year was estimated to be about 6.7% (Jeyaratnam 1990). Size of population at risk of pesticide exposure is largest in developing countries like Malaysia, since the majority of the economically active members of the population work in agriculture. Twenty five percent of labour force participation in Malaysia is encompassed by agricultural-based economic activity (Department of Statistics Malaysia 1991). The usage of pesticides is about 40,000 metric tons annually (O'Malley 1997).

Organic insecticides are compounds that have been used globally for pest control over 100 years. These are common agents of suicidal or accidental poisoning as a result of their availability and easy accessibility (Peter and Cherion, 2000). Organophosphate (OP) insecticides are the most widely used and most toxic pesticides in the world. Since 1980's, organophosphate compounds have been the principal means of agricultural-pest control throughout the world (Stephens *et al.*, 1995). Being the insecticides of choice in the agricultural world, they are the most common cause of poisoning among the pesticides.

The United States Environmental Protection Agency (EPA) reported that 80% of all hospitalisations from pesticides poisoning were due to organophosphate group. The World Health Organisation (WHO) estimated that more than three million cases of acute serious pesticide poisoning occured worldwide annually, the majority being caused by OP especially in developing countries. In third world countries, organophosphate poisoning is a major cause of morbidity and mortality and accounts for a large proportion of patients admitted into intensive care units (ICU). At the Christian Medical College and Hospital, Vellore, India, OP accounts for 12.1% of all ICU admission and 29.0% of poisoning. In another centre it accounted for 89.5% of the 107 patients admitted for poisoning. A similar picture was also observed in Pakistan where it accounted for 39.7% of cases admitted with poisoning and 16% of total admissions to the ICU (Peter and Cherion, 2000).

OPs are popular insecticides because of their effectiveness and lack of persistence in the environment due to their unstable chemical nature. Thus, they do not persist in body tissues or the environment as do organochlorides and subsequently have replaced DDT as the insecticides group of choice (Bardin *et al.*, 1994). The common mode of poisoning is ingestion of OP in an attempt to commit suicide, which is still prevalent in some countries (Peter and Cherion, 2000). This problem appears to be frequent in developing countries possibly due to the widespread availability of pesticides as a result of extensive use in agricultural communities and because many such countries also allow the sale of OP directly over the counter and have inadequate regulations controlling their use and storage. This will lead to indiscriminate handling and storage as a result of the lack of knowledge about the serious consequences of poisoning.

1.1: Pathophysiology of OP Insecticide Poisoning

Acetylcholine (ACh) is the neurotransmitter released by terminal nerve endings of all post-ganglionic parasympathetic nerves, and sympathetic and parasympathetic ganglia. It is also released at skeletal muscle myoneural junctions and serves as a neurotransmitter in the central nervous system. In human beings, the two principal cholinesterases are erythrocyte, or true cholinesterase (acetylcholinesterase) (AChE), which is found primarily in the nervous tissues and erythrocytes, and serum cholinesterase (pseudocholinesterase) (ChE), which is found in serum and liver. Normally the AChE rapidly hydrolyses ACh into inactive fragments of choline and acetic acid after the completion of neurochemical transmission. OP binds irreversibly to the AChEs and convert them into inactive proteins resulting in the accumulation of ACh at the above-mentioned receptors leading to over stimulation and subsequent disruption of nerve impulse transmission in both the peripheral and central nervous system. The OP-AChE complex may either be hydrolysed to yield functional AChE again or, depending on time, the OP component may undergo chemical change that prevents hydrolysis and reactivation of AChE, a process commonly referred to as ageing. Ageing is more common with some OP than others. The onset of symptoms and severity of acute OP poisoning is thus dependent on: -

1. the rate at which AChE is inactivated,
2. the rate of dissociation of inactivated AChE to release active enzyme,
3. the rate at which ageing occurs,

4. synthesis of new AChE by the liver (this takes weeks) (Jones and Proudfoot, 2000).

1.2: Aetiology of OP Poisoning

According to Peter and Cherion (2000), OP poisoning results either from suicidal or accidental poisoning or from occupational exposure during farming. A large proportion (68%) presents following suicidal attempts. Most patients are young (< 30 years) with a male preponderance. Occupational exposure and accidental poisoning accounted for 16.8 and 15.8% respectively.

1.3: Clinical Features of OP Poisoning

Clinical features of acute poisoning occur, almost always, within 24 hours of ingestion / exposure of the OP compound. In the initial stages, symptoms and signs may be of a non-specific illness like gastro-enteritis with vomiting, diarrhoea and abdominal pain. A high index of suspicion is therefore needed to make the diagnosis early. Skilled and prompt treatment can provide a good outcome for a potentially lethal condition. The clinical features can be broadly classified as secondary to the

a. muscarinic effect,

b. nicotinic effect, and

c. central receptors stimulation.

The end result may be multi-system manifestations involving the gastro-intestinal tract, respiratory system, cardiovascular system, nervous system, skeletal muscles, other organs and metabolic effects such as hypo- or hyperglycaemia.

1.4: Neurological Manifestations In Acute OP Poisoning

Much importance has been given to the neurological manifestations in OP poisoning as a large number of patients with acute exposure develop neuromuscular weakness requiring prolonged ventilation. Three different types of paralysis are recognised based largely on the times of occurrence and their differing pathophysiologies.

1.4.1: Type I Paralysis or Acute Paralysis

This occurs within 30 minutes to several hours after exposure along with the initial cholinergic symptoms. It is postulated to be the result of persistent depolarisation at the neuromuscular junction due to blockade of AChE. Features include muscle fasciculation, cramp, twitching and weakness. It has been claimed that these manifestations respond to atropine, although one would not anticipate atropine blocking the nicotinic effects of ACh (Peter and Cherion, 2000). Paralysis may also involve the respiratory muscles leading to respiratory embarrassment or arrest requiring ventilation. This may be further compounded by the bronchorrhoea that predisposes to pneumonia. Acute respiratory failure was observed in 33% of patients who presented with acute OP poisoning. A patient who survives 24 hours will usually recover.

1.4.2: Type II Paralysis or Intermediate Paralysis

This was first described in 1974 by Wadia *et al.* as Type II signs and subsequently characterised and termed as "Intermediate Syndrome" by Senanayake and Karalliede in 1987 (Van Der Hoek *et al.*,1998). It is a distinct clinical entity that develops after the acute cholinergic crisis and before the expected onset of delayed neuropathy. This was observed in Senanayake's series of 10 patients who on recovering from the cholinergic crisis developed the syndrome 24 to 96 hours after the poisoning. In 70% of patients respiratory insufficiency drew attention to the onset of the syndrome. Various degrees of cranial nerves palsies and proximal muscle weaknesses were observed while there was relative sparing of the distal muscle groups (Senanayake and Karalliede, 1987). De Bleecker *et al.* (1993) reported that the incidence of this syndrome varies between 8 and 49%. One of the earliest manifestations in these patients is the presence of marked weakness of neck flexion with inability to lift the neck from the pillow. This is a useful test to assess if a patient is likely to develop respiratory muscle weakness. The usual cranial nerves involved are those supplying the extra ocular muscles whilst cranial nerves VII and X are less frequently affected. Electromyographic (EMG) studies suggest a combination of pre- and post-synaptic dysfunction of neuromuscular transmission. This syndrome persists for about four to 18 days and most patients survive this unless infections or cardiac arrhythmias complicate the course. Electromyographic improvement precedes clinical recovery.

1.4.3: Type III Paralysis or OP-Induced Delayed Neuropathy (OPIDP)

This was observed in Vasilescu's series of 4 patients who had delayed neuropathy after 3-5 weeks of exposure to Dipterex, an OP insecticide in Bucharest, Romania (Peter and Cherion, 2000). He later discovered that clinical electrophysiological and nerve biopsy findings were compatible with a mixed distal sensorimotor (predominantly motor) neuropathy. The EMG features were suggestive of denervation and recovery was delayed up to 6 to 12 months (Vasilescu *et al.*, 1984).

1.4.4: Chronic Neuropsychologic, Neuropsychiatric and Other Neurologic Manifestations

Reports of possible chronic effects of OP exposure have appeared in the scientific literature for more than 50 years (Expert Panel on OP Sheep Dips, National Registration Authority for Agricultural and Veterinary Chemicals NRA). Researchers at the University of Melbourne and Prince Henry's Hospital presented brief case studies of 14 men and 2 women exposed to OP between 1.5 to 10 years. These subjects were suffering from schizophrenic and depressive reactions, with severe impairment of memory and difficulty in concentration. A limited field survey suggested that psychiatric disorders might be more common in fruit growing areas than in towns (Gershon and Shaw, 1961). A separate study compared schizophrenia incidence with OP sales in Australian fruit growing areas. It was found that schizophrenia was 1.9 times more common in two areas where sales were 7.9 times higher than the reference area. However, follow-up work could not link individual cases to OP insecticides use. Also, in a larger population of 5,034 growers there was no correlation between schizophrenia or depression, and pesticide sales. It was recognised that measures of exposure and effects were limited, which did not allow more than the detection of a gross effect (Stoller *et al.*,1965).

Stephens *et al.* (1995) described the various neuropsychiatric manifestations in chronic OP poisoning as chronic neurobehavioural symptoms including depression, irritability, confusion, chronic tiredness, apathy, headache, dizziness and emotional liability. Persistent subtle neuropsychologic impairment after an episode of acute poisoning may be more prevalent than previously thought. The demonstration in recent years of subclinical neurotoxicity, as many of the neurotoxicants in the environment can cause dose-related adverse effects through exposure too small to produce signs and symptoms that are evident in a standard clinical examination, could not discriminate between poisoned subjects from controls (Savage *et al.*,1988).

Chronic low-level exposure to OP has also been linked to an encephalopathy, with forgetfulness and other cognitive dysfunctions as chief complaints (So, 1997). Duffy *et al.* (1979) suggested that the long-term behavioural changes and long-term electrophysiological abnormalities do provide parallel evidence that OP exposure can produce long-term change in the brain function of monkeys and human.

Epidemiological data are limited on chronic neurological sequelae to acute OP poisoning and intermediate syndrome following recovery from acute episode of exposure with more serious complications such as laryngeal paralysis (Indudharan *et al.*, 1998). Aiuto *et al.* (1993) reported a prolonged bilateral vocal cord paralysis after accidental OP poisoning in a 3-year-old child who required prolonged ventilator support and recovered slowly.

Information about the effects of long-term, low-dose OP insecticide use was not initially available. Thompson and Stocks (1997) quoted a study by Dubois *et al.* in 1949 in which the typical presentation of low-dose OP poisoning on the illnesses and odd behaviours of Nazi nerve gas factory workers which is now regarded as the classic delayed type of toxicity syndrome. The patients displayed memory loss, tremor, fatigue, general weakness, and emotional lability for up to 4 months. Higher doses resulted in lengthy paralysis similar to that of botulinum poisoning. Thompson and Stocks (1997) later suggested a new variant of the three previously described types of toxicity syndrome. The primary findings are somnolence and bilateral vocal cord paralysis. The condition is short-term and can be managed by intubation and ventilator support. It may represent a halfway point between the well-documented acute onset cholinergic crisis phase and the more delayed intermediate type syndrome of OP poisoning.

The persistent central nervous system effects of OP were suggested by long-term difficulties with memory and concentration, and schizophrenic and depressive reaction (Rosenstock *et al.*, 1991). Stephens *et al.* (1995) showed that sheep farmers exposed to OP during sheep dipping performed significantly worse than controls in tests to assess sustained attention and speed of information processing after controlling for covariates. The farmers also showed greater vulnerability to psychiatric disorder compared to controls based on the General Health Questionnaire (GHQ) results. In another study, Beach *et al.* (1996) demonstrated significant reduction in scores of neurological tests (two point discrimination on the dorsum of the hand and foot, and mean calf circumference) of sheep farmers chronically exposed to OP compared to unexposed quarry workers as controls. Stokes *et al.* (1995) also showed significantly higher scores of mean vibration threshold for dominant and non dominant hands among chronically OP exposed pesticide applicators compared to scores of age-sex, and country of residence matched population controls. An earlier case report by Wagner and Orwick (1994) noted persistent hypertonicity of the extremities in a 12-week-old infant girl following a prolonged continuous exposure to low-levels of OP in the interior of a home since she was 11 week-old. He was able to demonstrate that the symptoms presented without cholinesterase depression but demonstration of exposure was made possible by the presence of alkyl phosphates in the infant's urine nearly 8 months after the application and unexpectedly high residue of diazinon were found in the home. Ames *et al.* (1995) provided some assurance that their findings in a study of 45 professional pesticide applicators using a variety of OPs had at least one episode of ChE inhibition but no symptom of central or peripheral nervous system and these effects do suggest that preventing acute OP poisoning may also prevent chronic neurologic sequelae.

1.5: Cholinesterase Estimation

Both AChE and ChE levels can be estimated to assess poisoning. These levels are markedly reduced in acute OP poisoning. While AChE levels correlate with severity at presentation, ChE levels do not. London *et al.* (1995) noted that AChE has a recognised association with exposure to organophosphates and is known to have longer lasting effect than ChE.

AChE and ChE have been used to monitor the extent of OP exposure (Gomes *et al.*,1997). However Coye *et al.* (1987) suggested that the inhibition of AChE as the better indicator of biological effects. The use of AChE as a useful biomarker for chronic pesticides toxicity has been reported by Mc Connell and Magnotti (1994). London *et al.* (1995) reported excellent sensitivity and specificity for AChE using a spectrofluorometric field kit. Symptomaticity may occur only with severe AChE depression. Hence, the measurement of pseudocholinesterase is more appropriate in chronic exposure to organophosphate compounds keeping in mind certain medical conditions that cause low serum cholinesterase levels such as parenchymal liver disease, congestive cardiac failure with hepatomegaly, metastatic carcinoma, malnutrition, dermatomyositis and acute infection (Peter and Cherion, 2000). AChE testing is used primarily to confirm the diagnosis of OP poisoning where a 25% or greater depression in AChE level is taken as a true indicator of OP poisoning. Areekul *et al.* (1981) found that gradual depletion of cholinesterase activity is apparently better than abrupt depression and a prolonged low-level exposure to OP compounds may diminish the cholinesterase activity to a low level in man without producing severe symptoms of toxicity. The routine monitoring of OP exposed workers using plasma or erythrocyte cholinesterase depression has been practised for many years to detect subclinical OP poisoning.

1.6: Neurobehavioural Testing

During the past 30 years, neurobehavioural methods have been used increasingly to investigate effects on central nervous system of exposure to neurotoxic chemicals. These methods assess the behavioural outcomes of toxic insult by measuring effects on aspects of cognitive functioning such as visuomotor skills, the ability to sustain attention, and higher level information processing abilities such as learning, memory, and logical reasoning. From the point of view of occupational health, these methods offer several advantages. They are non-invasive and therefore generally acceptable to workers, and they are also portable, and relatively inexpensive. More importantly, they provide a powerful tool for assessing the more subtle changes that may occur at lower levels of exposure in the absence of overt clinical symptoms and signs. Potential applications of these methods in occupational health include research, diagnosis, and screening. Neurobehavioural tests have been used extensively either in laboratory-based studies designed to identify acute reversible effects of neurotoxicant exposure and the levels at which these effects occur, or in large scale epidemiological type studies to investigate chronic effects of long term exposure. Applications in cross-sectional research studies, involving the comparison of performance of exposed and control groups, are well established (Spurgeon 1996; Stephens and Barker, 1998).

1.7: Justification of Study

This study attempted to evaluate the neurotoxic effects of occupational exposure to OP insecticides in chronically exposed tobacco farmers by conducting neurobehavioural testing. Many reports on workers chronically exposed to low levels of OP were unable to provide conclusive evidence of neuropsychological impairment in those workers. Neurobehavioural tests are used to study the effects of exposure to chemical and physical agents including pesticides. It is a tool of behavioural toxicology which draws on the disciplines of experimental psychology, behavioural pharmacology and behavioural brain research. Behaviour is the final product of nervous system activity. Therefore, any toxicant-induced changes in the nervous system may be reflected by behavioural changes (WHO 1986). The battery of tests to be administered in the study is the World Health Organisation recommended Neurobehavioural Core Test Battery (NCTB), which meets the following three criteria:

1. Statistically significant differences between exposed and non-exposed groups were detected in workplace research.
2. Technicians could, following minimal training, reliably administer the NCTB tests.
3. Materials and equipment were inexpensive and could be administered in remote settings.

The purpose of NCTB is to measure a broad range of neurobehavioural functions in 4 domains:

1. Psychomotor speed and dexterity
2. Memory
3. Mood
4. Visual spatial ability.

The test takes about 1 hour per subject with mostly pencil and paper tests.

1.8: Conceptual Framework

There are a few factors that have been identified to influence the usage of pesticides (Figure 1). The main factors are knowledge, attitude, and practice.

(a) Knowledge and Attitude About Pesticides

Knowledge about pesticides is attributed to education. One with formal education is more readily to understand instruction and able to read carefully about insecticides either from a book or poster. Informal education is through the explanation given by the mass media, agricultural workers, friends, or the retailer. Other factors that greatly influence knowledge is socio-economic status, where low-income farmers were unable to buy mass media equipment such as television, magazines, newspaper, and etc.

(b) Practice

Farmers will mix the insecticides together with other agrochemical product to yield a better effect in order to have better quality of tobacco leaves without thinking of the adverse effects.

(c) Government's Policy

The Pesticides Act 1974 is promulgated to monitor and control registration, labelling, sales, and pesticides residue in agriculture perhaps not being adequately enforced, especially in the state of Kelantan where smuggling activities occurring near the border. These need a collaborative effort to ensure adequate law enforcement done.

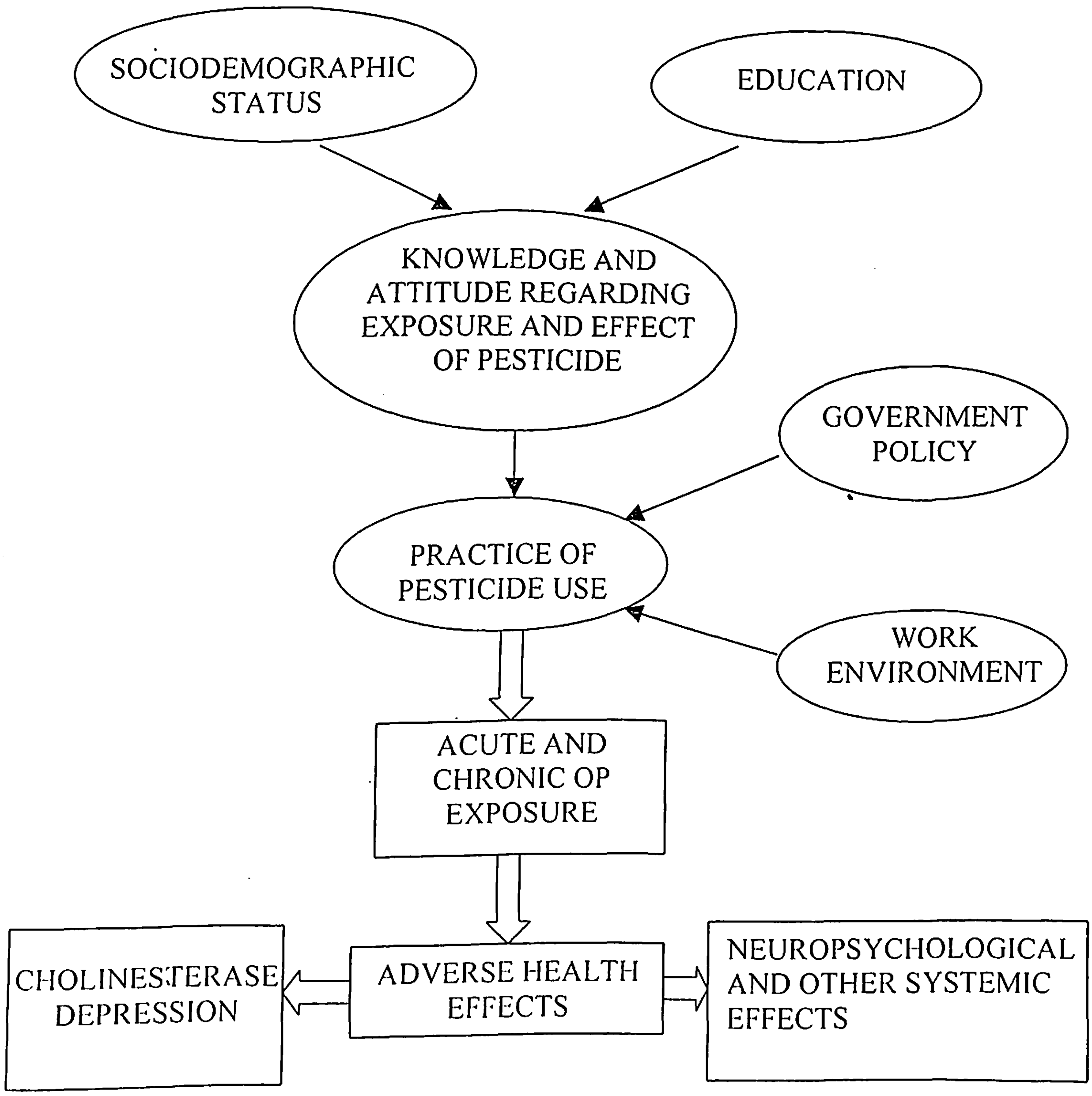
(d) Work Environment

When the farmers discovered that there are insects in the plants, there are tendency to use higher dosage of insecticides. Working environment also influence the work practice, for example; it is difficult to practice hand washing after pesticide use in a farm without washing facilities though the farmers are aware about the proper hygiene care regarding pesticide use.

(e) Adverse Effects on Health

Adverse health effects following chronic OP exposure are extremely diverse and we are focussing on the effects on the depression of ChE, neuropsychological effects, and other systemic manifestations such as muscular weakness and peripheral neuropathy.

Figure 1. Flow chart showing the conceptual framework of factors contributing to adverse health effects of pesticide use in tobacco farmers.



CHAPTER TWO

OBJECTIVES

2.1: General

To study the knowledge and practice of pesticide use and to assess the neurobehavioural effects of long-term exposure to OP insecticides in tobacco-growing farmers in Kelantan.

2.2: Specific

1. To compare symptoms related to chronic OP exposure between exposed farmers and non-exposed workers.

2. To compare the NCTB performance scores in exposed farmers and non-exposed workers.

3. To compare the ChE levels in exposed farmers and non-exposed workers.

4. To identify association, if any, between ChE and NCTB performance scores in exposed group.

5. To identify association, if any, between sociodemographic factors and NCTB

performance scores in exposed group.

6. To assess the knowledge about adverse health effects of pesticides and practice of safe behaviour related to pesticide use in farmers.

2.3: Research Hypotheses

1. Exposed farmers reported more symptoms related to chronic OP poisoning compared to non-exposed workers.

2. There is a difference in NCTB performance scores in exposed farmers and non- exposed workers

3. There is a difference in the ChE levels in exposed farmers and non-exposed workers.

4. There is an association between ChE and NCTB performance scores in exposed farmers.

5. There is an association between sociodemographic factors and NCTB scores in exposed farmers.

6. The knowledge about adverse health effects of pesticides and practice of safe behaviour related to pesticide use in farmers are good.

CHAPTER THREE

METHODOLOGY

3.1: Research Design

This is a cross-sectional comparative study designed to investigate (a) neuropsychological symptoms, (b) neurobehavioural performances, and (c) cholinesterase levels and (d) knowledge and practice of pesticide use in tobacco farmers chronically exposed to OP.

3.2: Sample Size

Sample size calculation was based on the prevalence of pesticides poisoning in agricultural workers (Peter and Cherion, 2000): 16%, and 1% background expected prevalence in non-exposed workers. The power of the study was decided at 80% with an α-value of 0.05. The calculated sample size, based on two proportions, was 30 for each group (exposed farmers and non-exposed workers). We decided to study 45 exposed farmers and 45 non-exposed workers.

3.3: Sampling Method

We developed specific inclusion and exclusion criteria to select our study samples. The inclusion criteria for detecting the exposed group include; (a) male gender, (b) age between 18 to 59 years, and (c) at least 2 years experience as farmers. There has been report that there are influences of age and sex on human blood cholinesterase activity (Sidell and

Kaminskis, 1975). Person younger than 15 years were excluded from the study because the adult neuropsychological test battery was not appropriate for them, and persons older than 60 were excluded because more extreme "normal ageing" effects on neuropsychological performance would be expected for such subjects (Savage *et al.*, 1988). The exclusion criteria include: (a) medical histories of pesticides poisoning within 3 month prior to the study [which could be associated with sub acute, irreversible sequelae and subclinical intoxication of workers exposed to pesticides occurs during every workday (López-Carillo and López-Cervantes, 1993)], (b) disease or injuries to the central nervous system (including trauma with period of unconsciousness totalling more than 15 minutes), (c) history of learning disability or congenital defects of the central nervous system, and (d) history of alcohol, narcotic, or other drug abuse. Similarly, in selecting the non-exposed group, the following inclusion and exclusion criteria were applied: Inclusion criteria (a) male gender, (b) age between 18 to 59 years, and (c) no exposure to agrochemical products. Exclusion criteria (a) medical history of central nervous system illness, (b) history of head trauma, and (c) residing nearby tobacco-planting area. The exposed group comprised of tobacco-growing farmers in Bachok District, Kelantan. A sampling frame was constructed from database of annual census data for tobacco farmers available from National Tobacco Board (NTB) of Malaysia maintained by the Kelantan Head Quarters for the district of Bachok, one of the ten districts in the state of Kelantan. In 2000, there were altogether 6819 tobacco-growing farmers registered in the Bachok District, in which 4069 (59.7%) were male and 2750 (40.3%) were female. From the list of 4069 male tobacco-farmers, 2650 (65.1%) fulfilled the inclusion and exclusion criteria. We non-randomly selected 45 farmers who fulfilled the inclusion and exclusion criteria. We contacted each of the 45 farmers through the local NTB offices in Bachok District. The farmers were briefed about the study and their informed consents to participate in the study were obtained (Appendix A). All farmers selected registered to participate in the study, i.e. a 100% response rate (Appendix B). We also screened supporting staff from various departments in HUSM as potential non-exposed subjects. Altogether, 45 supporting staff were recruited on volunteer basis. They were briefed about the study and their informed consents were obtained (Appendix A). All supporting staff selected registered to participate in the study, i.e. a 100% response rate (Appendix B). The study was done at a time when farmers in Bachok had not been involved in spraying of pesticides for at least 2 months to ensure that any effects observed were not related to recent exposure (August and September).

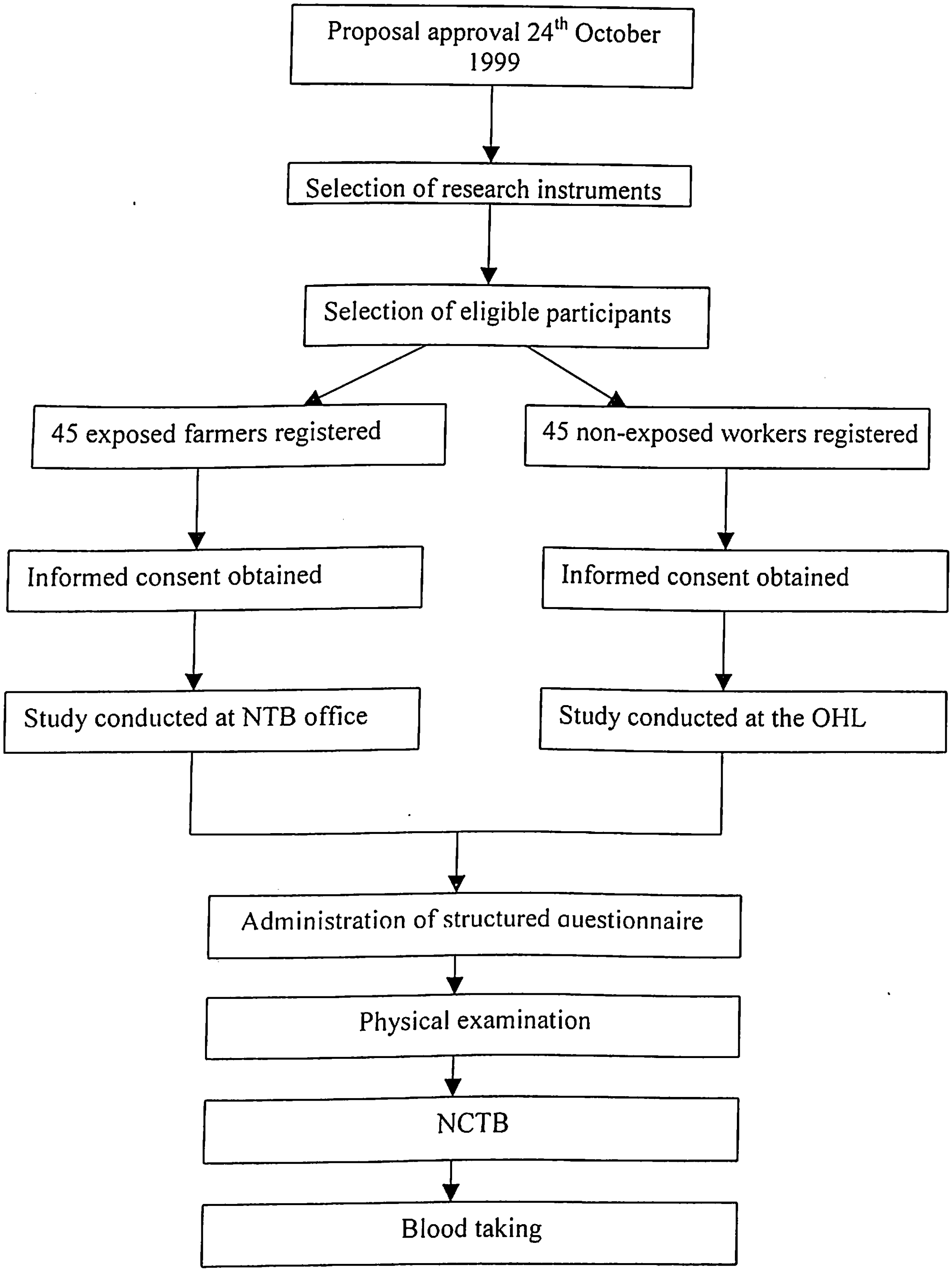
3.4: Research Protocol

The research proposal was approved on 24th October 1999 and the selection of research instruments was commenced. These include (a) structured questionnaire, (b) physical examination, (c) NCTB, and (d) ChE. The Research and Ethical Committee, School of Medical Sciences, USM, Kelantan Health Campus approved this study. Selection of eligible participants was carried out to allocate subjects into exposed farmers and non-exposed workers. The study was conducted at a local NTB office for tobacco farmers and in the Occupational Health Laboratory (OHL), Department of Community Medicine, School of Medical Sciences, USM, Kelantan Health Campus for the non-exposed workers. Figure 2 shows the Gantt chart of study progress and Figure 3 illustrates the flow of the study.

Figure 2. Gantt Chart showing progress of dissertation

No	Task Name	1999	2000	2001
1	Research Proposal	2/6 22/10		
2	Questionnaire Design	1/11	12/4	
3	Introduction		1/5 6/6	
4	Data Collection		1/8	30/3
5	Data Analysis			1/4 30/4
6	Report Preparation			1/5 30/7
7	Submission of First Draft			31/7
8	Dissertation Revision 1			18/11
9	Dissertation Revision 2			24/11
10	Dissertation Revision 3			26/11
11	Submission of Final Dissertation			29/11

Figure 3. Flow chart of the study



3.5: Research Instruments

3.5.1: Structured Questionnaires

Structured questionnaire (Appendix C) included the following:

(a) Section A: Sociodemography (14 questions)

(b) Section B: Other Chemical Exposure (4 questions)

(c) Section C: General Health Questionnaire (10 questions)

(d) Section D: Neuropsychological Symptoms Questionnaire (36 questions)

(e) Section E: Safe Practice Of Pesticide Use (20 questions)

(f) Section F: Knowledge About Adverse Health Effects of Pesticide (4 questions)

3.5.2: Physical Examination

Each participant receives a physical examination to elicit any evidence of systemic illness before the conduct of NCTB (Appendix D).

3.5.3: NCTB

Seven tests from the WHO NCTB (Appendix E) were performed by the exposed farmers at the NTB office and non-exposed workers at the OHL, Department of Community Medicine, School of Medical Science, Universiti Sains Malaysia, according to the operational guide of WHO (WHO 1986). Profile of mood states (POMS) was used to assess affect in the domain of tension-anxiety, depression, anger-hostility, vigour, fatigue and confusion. Simple reaction time was used to estimate attention and response speed, digit symbol for the perceptual-motor speed test, digit span forward and backward for the auditory attention and memory test. Santa Ana manual dexterity (preferred hand, non-preferred hand) to estimate manual dexterity, Benton visual retention for the visual perception and memory test, and pursuit aiming for assessing motor steadiness. A brief description of the tests with functional domain within parentheses is listed below:

1. *POMS (affect).* The subject rates himself on a scale from 0 to 4 about feelings experienced during the previous seven days. These include 65 items and provide a six-point mood profile: tension, depression, anger, vigour, fatigue and confusion. The profile of mood states was a sensitive indicator of neurotoxicity in a previous study of workers exposed to inorganic lead (Maizlish *et al.* 1995).

2. *Simple reaction time (psychomotor performance).* Simple reaction time measures simple visual reaction time and it requires sustained attention by the subject. The subject responds to a red light stimulus presented at delays from one to ten seconds in a 2 cm window of a reaction timer by immediately depressing a small yellow button with his index finger. The mean simple reaction time (ms) of 64 trials is the response variables. Stephens *et al.* (1995) had showed significantly slower reaction time in farmers who had been exposed to organophosphates.

3. *Digit symbol (perceptual motor-speed).* Digit symbol measures perceptual speed. The subject is presented with a key at the top of the page number, one to nine displayed with their respective matching symbols. Below are blank blocks with digits above. The subject