CHAPTER 2

LITERATURE REVIEW

2.1 Pesticide definition and classification

Pesticides are chemical, biological and physical agents used to kill and control pests, insects, animals and weeds in agricultural area. The Food and Agricultural Organization (FAO) defined a pesticide as any substance or mixture of substances intended for preventing, destroying or controlling any pests including vectors of human or animal diseases, unwanted species of plants or animals causing harm during the production, processing, stroage, transport or marketing of food and agricultural commodities (FAO, 1968).

Pesticides are classified into four major categories due to chemical structure, Organocholrine pesticides (OCPs), Organophosphate pesticides (OPs), Carbamate group (Carb) and Pyrethroid group (Pyr) (WHO, 1996; 1997).

Otherwise, pesticides are also classified by type of use, such as insecticide, fungicide, herbicide and others. Insecticide chemical agents are used to kill the unwanted insect pest species. Herbicide substances kill weed plants or inhibit their growth. The selective herbicides are designed to affect weeds without permanently harming crops, while the non-selective one, generally toxic, are used to clear all plants. Fungicide substances are used to prevent infection by or to destroy fungi and

are made from sulfur or copper compounds, organic salts of iron, zinc and mercury and other chemicals. Fungicides are used on seeds, growing timber crops, fabrics, and for human and animal fungal disease (Longman, 1990).

Pesticides are used to increase crop yield, improve the quality of crops and gain the good apprearance of products. However, concern about the danger of their insecticide residues in the ecosystem and in foodstuffs. Such concern has led some countries to move strict governmental regulation of pesticide use; for example by using less toxic compounds and replacement of toxic insecticides that persist in the environment by compound that break down more qucikly into non-toxic form.

However, only organophosphate and carbamate compounds are inhibitors of cholinesterase enzymes and are widely used as insecticide for this reason. Signs and symptoms of pesticides poisoning are usually due to the powerful inhibition of cholinesterase enzyme in the brain.

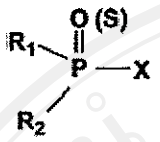
2.1.1 Organophosphate pesticides

Organophosphate pesticides are generally used in agricultural field and mostly used for their insecticidal activity to prevent weeds, improve quality of products and increase agricultural yield. The major compounds of toxicological interest are esters and thiols derived from phosphoric, phosphonic, phosphinic or phosphoramidic acid. The basic chemical structure of OPs is displayed in figure 1 (Miguel, 2002). The OPs

exert their main toxicological effects through non-reversible phosphorylation of esterases in the central nervous system (Aldridge, 1972; WHO, 1986).

The toxic effects of organophosphate pesticides are caused by an inhibition of acetylcholinesterase enzyme (AchE) activity, which results in neurotransmitter inactivation. The accumulation of acetylcholine at the cholinergic synapses results in peripheral and central nervous system effects. The cholinesterase levels in red blood cell (Rbc) and plasma are indicators of the organophosphate pesticide exposure. Death is usually due to the powerful inhibition of acetylcholinesterase enzyme in brain (Kai, 2004; Michele, 2002).

Most organophosphate pesticides are highly toxic substances. Acute effects of pesticide poisoning included nausea, vomiting, abdominal pain, numbness, tremors, fatigue, headaches, excessive salivation, diarrhea, generalized weakness, respiratory problems and blurred vision. The psychological effects may be present as anxiety, depression, irritability and restlessness. The chronic effects of pesticide poisoning are usually found in occupational workers such as farm workers and pesticide industry workers. The chronic effects have been linked to delayed onset peripheral neuropathies, neuropsychological changes and neurobehavioral changes (Lorann, 2002).



P = Phosphorus atom

R1 = aryl or alkyl group

R2 = aryl or alkyl group

O = Oxygen atom

S = Sulphur atom

X = aromatic, aliphatic or heterocylic group

Figure 1 Chemical structure of organophosphate insecticide

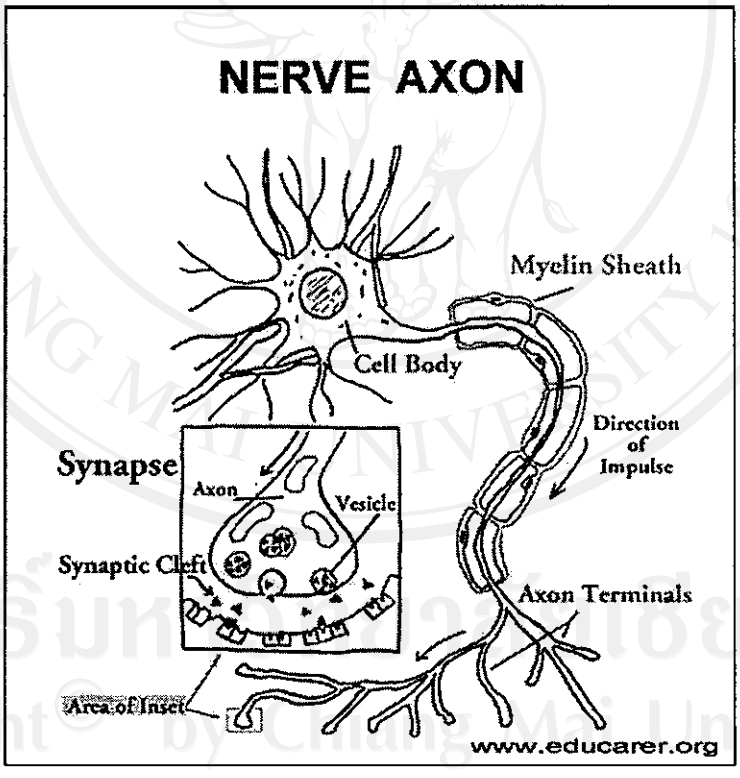


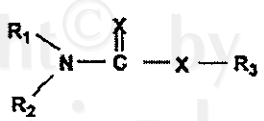
Figure 2 Cholinesterase enzyme released from axon terminals.

(Source: www.educarer.or)

2.1.2 Carbamate pesticides

Cabamate pesticides are also widely used in agriculture and cause the widest range of biocide activities. The structures of biologically active carbamates are shown in figure 3. In the carbamate compound structure, the X could be oxygen or sulphur. R1 and R2 are usually organic radicals or hydrogen. If R2 is hydrogen and R1 is methyl, carbamate exhibits insecticide activity. If R1 is an aromatic group the compounds are used as herbicides, but will be used as fungicide when R1 is a benzimidazole moiety. R3 group is mostly an organic radical or metal. (Miguel, 2002). Carbamate pesticides are also cholinesterase inhibitors which act to reversibly inhibit target esterases.

Carbamate and organophosphate pesticides are inhibitors of acetylcholinesterase and profuse the similar symptoms. Nevertheless, organophosphates and carbamates differ in the stability of the complex with acetylcholinesterase. Organophosphate pesticides could be able to phosphorylate serine residues of ChE in a non-reversible way while the carbamylation of carbamate compound is less stable. The typical decarbamylation time will be between 30 to 40 minutes.



C = Carbon atom
N = Nitrogen atom
R1 = Organic radical or hydrogen
R2 = Organic radical or hydrogen
R3 = Organic radical or metal
X = Oxygen or sulphur

Figure 3 Chemical structure of carbamate insecticide

2.2 Pesticide hazard classification

WHO classifies most pesticides by common name in terms of their potential human health effects. The criteria recommended by WHO are developed from a detailed examination of toxicoloigcal data of pesticide active substances and preparations and experience gained from their pratical use (Bates, 2000). These classifications are usually based on the acute oral LD_{50} levels. LD_{50} is based on experiments with animals and is the number of mg of toxicant per kg of body weight required to kill 50% of a large population of test animals.

Table 1 showed pesticide classification according to WHO hazards and guidelines. Pesticides hazardous generally belong to a chemical family and their LD50 on which general health effects.

Table 1 Pesticide Classification by WHO Hazard and Guidelines

Class	LD_{50} for the rat (mg/kg body weight)	
	Oral	
	Solids	Liquids
Ia = extremely hazardous	5 or less	20 or less
Ib = highly hazardous	5-50	20-200
II = moderately hazardous	50-500	200-2000
III = slightly hazardous	500-2000	2000-3000
O = unlikely if used safely	over 2000	over 3000

Source: The International Program of Chemical Safety. The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 1996-1997. WHO/PCS/96.3.

2.3 Pesticide application in agricultural fields

The amount of pesticide use in Thailand had increased year by year as shown in Table 2. Total amount of imported pesticides was 29,189 tons in the year 1994 and 66,434 tons in year 2002. Approximately 50% of all imported pesticide were herbicide, roughly 25% were insecticide and almost 12% were biopesticide and fungicide. The proportion of pesticides imported were nearly consistent in every year. However, they could be ranked from the high to low proportion as herbicide, insecticide, biopesticide, miticide, rodenticide, molluscicide, nematocide and fumigants.

The most common herbicides were phenoxy herbicides, triazines, amides, carbamates herbicides, dinitroanillines, urea derivatives, sulfonyl ureas, bipiridils and uracil. The generally used insecticides were organochlorine, organophosphate, carbamates, pyrethroids and botanical and biological products (Surasak, 2003; Thamarat, 2003).

Table 2 Amount of imported pesticide in Thailand during 1994 – 2002

Year / Type of use	Amount of pesticide (tons)								
	1994[1]	1995[1]	1996[1]	1997[1]	1998[2]	1999[2]	2000[2]	2001[2]	2002[2]
1. Insecticide	7,730	7,708	10,560	14,476	12,543	12,543	19,526	12,532	16,655
2. Herbicide	15,386	16,108	19,954	23,536	22,459	15,108	27,640	29,714	32,141
3. Biopesticide and Fungicide	15,386	7,065	6,937	6,447	5,820	-	7,204	7,392	7,768
4. Miticide	5,621	404	520	384	237	235	157	274	296
5. Rodenticide	469	98	86	92	-	-	216	141	89
6.Molluscide and Nematocide	129	46	36	216	72	46	150	226	80
7. Fumigants	37	345	50	82	219	190	286	570	787
Total	29,189	31,774	38,143	45,233	41,350	28,122	55,179	50,849	66,434

Source : 1. Report of environmental situation in Thailand, Ministry of Science and Technology, Thailand (1994-1997).

2. Ministry of Agriculture, Thailand (1998-2002)

Table 3 Area for planting in Thailand during 1994 – 1999.

Year	Area for planting in Thailand (rai)				
	Rice	Beans	Orchard	Vegetable and flower	Total area
1994	68,336,567	32,228,127	20,998,989	931,164	131,270,893
1995	68,320,651	32,130,516	21,638,428	937,789	131,833,288
1996	68,292,753	32,011,185	22,318,991	957,937	132,478,570
1997	67,457,556	31,119,785	23,131,363	959,523	131,819,506
1998	66,695,947	30,101,204	24,132,029	961,182	131,107,608
1999	65,914,065	29,051,965	25,079,047	961,792	130,393,525

Source: Department of Agriculture, Ministry of Agriculture and Cooperatives 2002.

Table 3 showed that the total area used was unchanged, whereas amounts of pesticide were increased each year. However, many factors affected the usage of pesticides including pest resistance, misuse, chemical antagonist and other (Keifer, 2000).

The document of pesticide situation in Thailand showed that 13 different pesticide products were found in the small community. About one third of them belonged to class Ia and Ib pesticides (extremely and highly hazardous) including methyl parathion, alachlor, methamidophos and methomyl. Another third were in class II (moderately hazardous) including highly poisonous paraquat and the organochlorine endosulfan, which is carcinogenic and a possible endocrine disruptor in animals (Areekul, 2003). Among 202 farmers, they used an approximate total of 387,130 liters pesticide per each year. That was about 1,916 liters of exposure for each farmer in a year. (Namrat, 2002)

Table 4 The twenty highest presentage of pesticides found in agricultural products during 1993 -2002.

No.	Chemical Name	Classification	No.	Chemical Name	Classification
1	Methamidophos	Organophosphate	11	Malathion	Organophospahte
2	Chlopyrifos	Organophosphate	12	Pirimiphos–methyl	Organophosphate
3	Dimethoate	Organophosphate	13	Dicofol	Organochlorine
4	Monocrotophos	Organophosphate	14	Methyl parathion	Organophosphate
5	Mevinphos	Organophosphate	15	Tetradifon	Organochlorine
6	Cypermethrin	Pyrethroid	16	Tetrachlorvinphos	Organophosphate
7	Carbaryl	Carbamate	17	Carbendazim	Carbamate
8	Methomyl	Carbamate	18	Mancozeb	Carbamate
9	Dicrotophos	Organophosphate	19	Zinep	Carbamate
10	Endosulfan	Organochlorine	20	Abamectin	Avermectin acaridides

Source: Department of Agriculture, Ministry of Agriculture and Cooperatives 2002.

The first five imported pesticides between years 1993 - 2002 are methamidophos, chlopyrifos, dimethoate, monocrotophos and mevinphos. All of them were organophosphate pesticides, especially methamidophos, mevinphos and monocrotophos which are classified by EPA as the class I compounds. They are also classified in class Ia by WHO classification. Organophosphates' mode of action in insects and mammals is by decreasing the activity of an enzyme important for nervous system function called acetylcholinesterease (Hussain, 1987). Signs and symptoms of acute exposure to organophosphate or cholinesterase inhibiting compounds may include these signs, tingling sensation, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision and difficult breathing, while very high doses may result in unconciousness, incontinence and convulsion or death (HSDB, 1990).

2.4 Impact of pesticide exposure on human health

WHO and the United Nations Environmental Program (UNEP) estimated that one to five million cases of pesticide poisoning occur among agricultural workers each year with about 20,000 fatalities. The major occurences were found in developing countries (WHO, 1990; Rosenstock, 1991; Pimental, 1992). Organophosphate and carbamate pesticides, anti-cholinesterases, were the most common causes of pesticide poisoning. Most public concern about the exposure to anti-cholinesterase, organophosphate, carbamate groups is because of the known potential for acute effects and the possibility of long term effects (Ray, 1998; Buranatrevedh, 2003).

Pesticide could enter the human body through 3 pathways, absorption, inhalation and ingestion. Farmers and pesticide industry employees are major worker groups who are highly exposed to pesticide. Generally, most occupational exposure in farmers occurs through the dermal route in the various stages of farming for example mixing, loading, spraying, applying pesticide and harvesting crops. (Buranatrevedh, 2003; Murphy, 2002) However, there are many factors that related to individual health effect of pesticide exposure including physico-chemical properties, solvents, impurities, duration and route of exposure, individual variation in metabolic, sequestration and excretory processes, age, gender, concurrent medications, and cholinergic status (Karallieddea, 2003)

Many occurrences of pesticide exposure are occur in the common body areas such as arms, hands, face, neck, nose and back. Pesticide could penetrate skin and cause systemic exposure and mostly acute illness and death cases reported from percutaneous absorption of pesticides particular through damage skin.

2.4.1 Effect of high dose exposure

Acute intoxication with pesticide produces a complex mixture of muscarinic (autonomic effectors cells) and nicotinic (muscular junction, autonomic ganglia and adrenal medulla) signs which vary in relative severity with target organ tissue, dose and agent (Marrs, 1993; Gils, 2004). An intermediate syndrome has been described as a late complication of some cases of severe acute poisoning (Senanayake, 1987). It is characterized by a specifically proximal muscle weakness lasting 5-18 days which can lead to respiratory failure. A further effect produced by some organophosphate is

organophosphorus ester-induced delayed polyneuropathy (OPIDPN) (Namrat, 2003). This is characterized by a delayed onset 10-14 days after exposure (Richardson, 1995). The lesion is a highly selective distal axonopathy affecting the distal axons of sensory, motor and autonomic neurons and the longer central tracts.

Organophosphate insecticides deactivate acetylcholinesterease enzyme, thereby destabilizing neurotransmission at synaptic nervous systems. N-methyl carbamate insecticides also deactivate acetylcholinesterase reversibly rather than permanently. Symptoms of organophosphate and carbamate poisoning are identical and equally severe but carbonates poisoning generally has shorter effect. Signs and symptoms include bradycardia, dyspnea, wheezing, nausea, vomiting, diarrhea, ocular meiosis, fasciculations, muscle weakness and hypersecretion (Ray, 1998).

Pesticide can irritate skin and cause dermatitis, allergic contact dermatitis, including photodermatitis and porphyria cutaneatarda. Exposure to methyl bromide, dichloropropene, paraquat and diquat, sulfur, ziram and benomyl might also cause dermatitis. Mancozeb, maneb, zinab, ziram, triforine and sulfur can cause dermatitis. Paraquat and diquat which are generally applied in farm can cause skin burns and severe fingernail damage (Buranatrevedh, 2003).

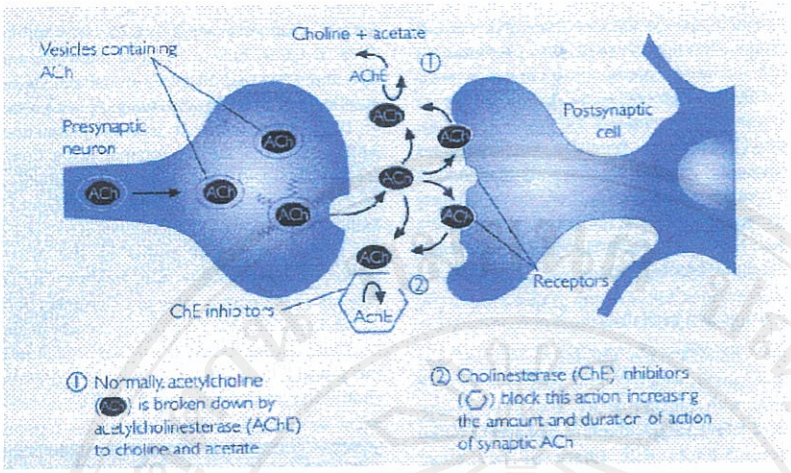


Figure 4 synaptic cleft releases acetylcholine and conducts signals from pre synaptic cell to post synaptic cell (Source:www.ashingtonhigh.northumberland.sch.uk)

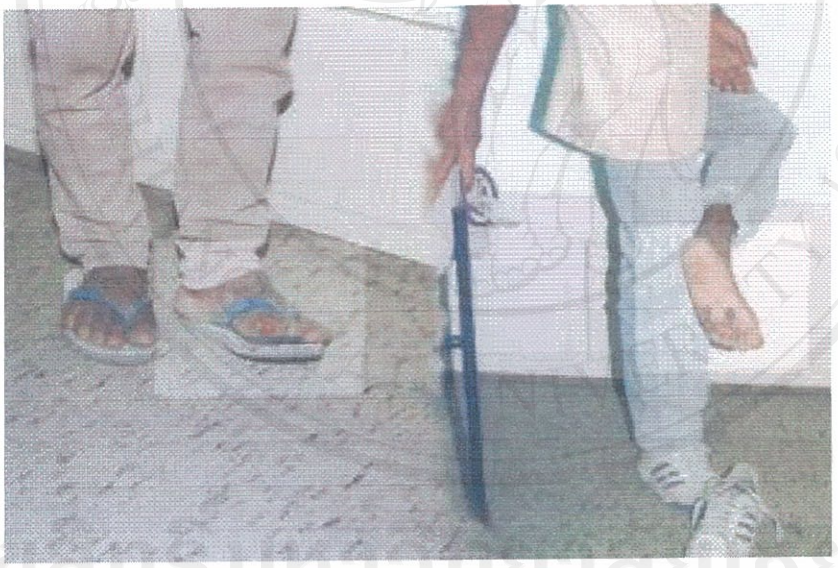


Figure 5 Skin burns and severe fingernail damage in high dose exposure to paraquat of a Thai farmer (Source: www.cadi.ph/ sustainable_agriculture.htm)

2.4.2 Effect of low dose exposure

Numbers of studies in non-occupational pesticide exposure found that residents living in agricultural area had significant public health consequences. Acute symptoms were investigated in non-occupational exposure to organophosphate insecticides in rural El Salvador. The 2-week prevalence of several acute symptoms were found and associated with living with a farmer who had recently applied methyl parathion. The odds ratios were 2.1 for cramps in limbs, 2.3 for feeling dazed and 2.5 for eyes tearing. The results suggest that living in areas where pesticides are used may represent an environmental concern, especially for children (Leonard and Lucas, 1999).

Residents in California who live in agricultural area were exposed to paraquat drift. They reported a prevalence of illnesses such as cough, diarrhea, eye irritation, headache, nausea, rhinitis, throat irritation, trouble breathing, unusual tiredness and wheezing. The odds ratios were 5.9 for diarrhea and 3.10 for nausea. (Ames et al., 1993) Residents of a cotton-growing community in California have experienced significant increases in prevalence of symptoms associated with a mixture of organophosphate pesticides and others (Scarborough et al., 1989). The Kibbutz residents exposed to organophosphate pesticide spray drift near their houses showed a significant dose response relationship between levels of urinary organophosphate metabolites and unsolicited complaints of at least one symptom (Richter et al., 1992).

Many studies of pesticide monitoring in farm communities and health effects in Asian region were conducted in several countries. Generally, investigators observed and collected pesticide use and other data by using an interview form which was developed and recommended by FAO as shown in Figure 6 (FAO). Farmers were educated and trained to fill in information about health effect and pesticide using in farm by themselves. The numbers of mild, moderate and severe signs or symptoms were totaled as well as the illness category - a mild, moderate, severe or non- illness event.

In Cambodia, many pesticides banned or highly restricted in most of the world were found. The majority of farmers mixed 3 to 4 different pesticides together for a single operation and some of them mixed up to 10. Pesticide sprayers reported an average of 12 adverse effects ranging from 3 - 22 of signs and symptoms. Eighty four percent of farmers had dizziness and 81% had headaches. They also had included chest pain (58%), stomach cramps and tremors (52%), nausea (48%), muscle twitching (48%), ataxia or staggering (35%) and vomiting (29%) (Makarady and Seng Horng 2002).

In Vietnam, 41 percent of 480 farmers had used highly toxic products such as methamidophos and most of them were poisoned by pesticide solution through spraying pesticide, wet clothing, hands and feet contact. On average, 6.7% of farmers experienced mild signs and symptoms directly after spraying while only 1.8 and 0.04% had moderate and serious signs and symptoms. 17.5 percent had moderate sign as ataxia-staggering and another 2 percent had vomited on site (Nguyen, 2001).

Eighty-eight percent of 60 Indonesia farmers were soaked with pesticide after applying pesticide and agrochemical by using backpack spray unit (Setyoko and Wienarto, 1999).

In Thailand, the study of pesticide and health effects in Thai farmers was investigated in 149 farmers. All of farmers were interviewed for poisoning and found that 4.9 percent had signs and symptoms of pesticide poisoning of which 3.6 percent were mild, 1.3 moderate and 0.01 serious of complaining. Signs and symptoms of every pesticide poisoning describe probable the cholinesterase depression by low dose exposure (Namrat and Tianponkrang, 2002).

The low dose of pesticide exposure effects to human health and approximately 4.9 to 80 percents of investigated farmers had sign and symptoms of pesticide poisoning such as dizziness, headache, chest pain, vomiting, stomach cramps tremors, nausea, muscle twitching and ataxia or staggering (Makarady and Seng Horng, 2002; Setyoko and Wienarto, 1999; Namrat and Tianponkrang, 2002). The most illness categories were mild to moderate event. Low dose exposure did not cause illness or death, but the number of poisoned farmers with of mild or moderate sign and symptom and use of banned pesticide indicate the further increasing poisoning cases.

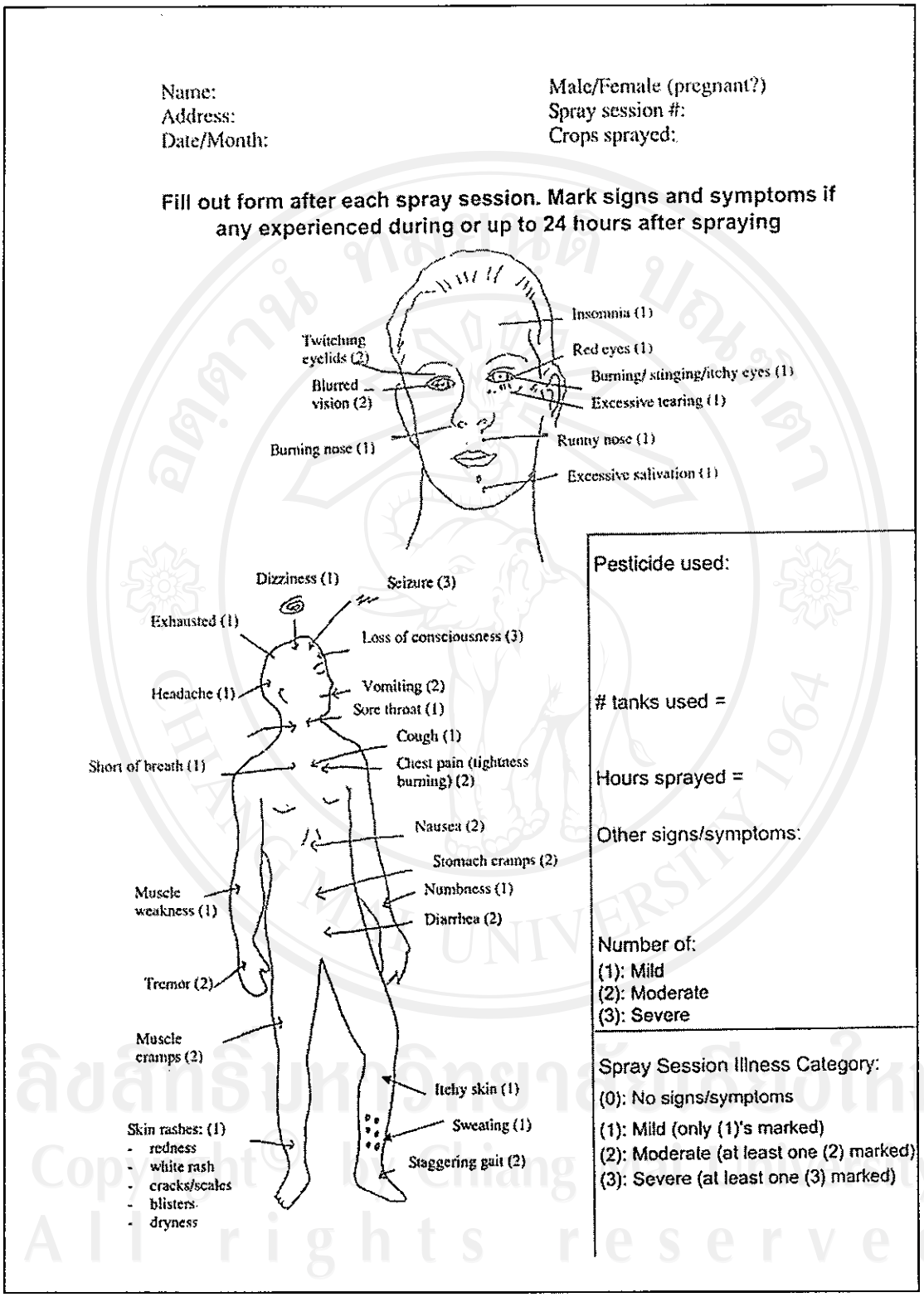


Figure 6 The FAO recommended interview form for health impact assessment

2.5 Cholinesterase enzyme activities and genetic variants

Biomarkers of pesticide exposure are important in monitoring, identification and interpretation of pesticide poisoning. Biomarkers are an indicator of internal dose, amount of chemical exposure that resulted in absorption into the body. The ability to accurately measure biomarkers of exposure depends upon an adequate understanding of the chemistry and toxicology of the substance under consideration. Organophosphate and carbamate pesticides share a common mechanism of toxicity, through inhibitory effects on cholinesterase enzymes in the nervous system. Cholinesterase monitoring has been used worldwide and is recommended by WHO as a means of illness prevention for workers exposed to organophosphate pesticides. Cholinesterase monitoring has been recognized in the scientific community as a legitimate means of protecting workers engaged in manufacturing, formulation and applying organophosphate pesticides and to a lesser extent, carbamate pesticides (WHO 1990).

Two enzymes were found in humans capable of hydrolyzing esters of choline. Acetylcholinesterase enzyme (AchE) is found in red blood cells and the region of cholinergic nerve fibers, especially in the synaptic cleft. Another enzyme is butylrylcholinesterase enzyme (BuchE), synthesized in the liver and generally found in liver, plasma, kidney and intestine. Both enzymes are responsible for the hydrolysis of cholinester and other forms.

Many compounds, such as physostigmine, neostigmine, sarin and others including organophosphate and carbamate pesticides, inhibit the enzyme reversibly by either binding with the esteratic site or anionic site.

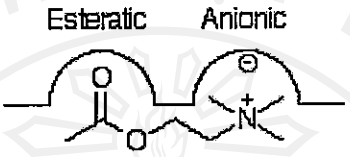


Figure 7 Esteratic and anionic site of acetylcholinesterase enzyme

(Source: http://bnlstb.bio.bnl.gov:8000/disk$3/giles/figs/2.GIF)

Studies of AChE inhibition were conducted in vivo and found the duration of inhibition of the enzyme is 3-4 hours for organophosphates, such as dimethylphosphate (DMP), dimethylthiophophate (DMTP), diethylphophate (DEP) and diethylthiophosphate (DETP) (Hatjian 2000), the resultant phosphorylated enzyme being extremely stable. These compounds act at the esteratic site, the tetrahedral geometry of the organophosphates resembling the transition state for the acetyl-ester hydrolysis. Stability of these complexes is enhanced by ***aging***, by which one of the phosphonate alkyl groups is lost with many of these agents. Significant regeneration of the enzyme is not observed and synthesis of new enzyme is required. The mechanism of action of ACh, the carbamyl esters and the organophosphates is essentially identical. Only regeneration of the active enzyme differs (Davis 1997, Gils 2004).

The assay method is based on the inhibition of acetylcholinesterase by organophosphate and carbamate pesticides. The thiocholine produced in the enzymatic reaction reacts with the nitrobenzoic acid and yellow color is released. The absolute absorbance (ΔAbs) was calculated and multiplied by the factor including extinction coefficient (ε), dilution factor (df) and incubation time (T) as shown in formula below (Ellman 1957).

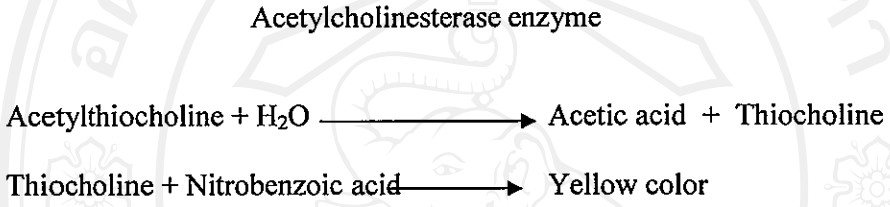


Figure 8 Hydrolysis of acetylcholine

$$\Delta Abs = AbsTest - AbsBlank$$

ΔAbs = *Absolute absorbance*

AbsTest = *Absorbance in Test tube*

AbsBlank = *Absorbance in Blank tube*

$$Activity = \frac{\Delta Abs}{13600} x \frac{TV}{SD} x \frac{1}{time}$$

Activity = *Cholinesterase activity (μmole/min)*

ΔAbs = *Absolute absorbance*

TV = *Total volume of enzyme analysis (ml)*

SD = *Sample dilution (ml)*

Time = *Incubation time (min)*

However, cholinesterase activity is dependent on its genetic variant, E1 and E2 Loci. Only one gene for human cholinesterase is located on the long arm of chromosome 3 at q21-25. All cholinesterase genotypes must arise from this locus. Abbreviations can be simplified by calling homozygotes UU, AA, SS, FF, KK, JJ and HH. Heterozytes can be abbreviated to show two of these alleles, for example UA for usual-atypical or UF for usual-silent. The genetic variant often found in patients who have abnormal response to succinylcholine is atypical cholinesterase. Silent cholinesterase has 0-2% of normal activity. The fluoride variant was found and sodium fluoride was used as inhibitor to identify the AF phenotype (Whittaker 1961).

2.6 Paraoxonase enzyme activities and PON1 gene

Paraoxonase or aryldialkylphosphatase (E.C. 3.1.8.1) is in A-esterase group which hydrolyzes organophosphate, carbonates and aromatic carboxylic esterase. Paraoxon is the substrate commonly used to measure enzyme activity. It requires Ca^{2+} for activity and stability, which distinguishes it from other enzyme in the A-esterase group such as diisopropylfluorophosphatase (DFPase) which require Co^{2+}, Mn^{2+} or Mg^{2+} for activity.

Paraoxonase enzymes are widely distributed among animals and can be present in many tissues, particularly liver, kidney, small intestine and serum. In situ hybridization studies have shown PON1 gene localized at q 21-q22 on the long arm chromosome 7 in human beings (Mackness 1998).

Paraoxonase activity is inhibited by sulfydryl reagents and this inhibition is reversed by cysteine. Thus a cysteinyl residue could be an essential component of the active site. The hypothesis were proved and found that –SH is required for the tertiary structure to maintain the active site residues in their optimal spatial arrangement. Studies of paraoxonase hydrolysis reaction by using purified human PON-1 at pH 10.5 were found that paraoxon is hydrolyzed with an initial burst-activity component with binding paraoxon followed by ordered release of p-nitrophenol followed by diethy phosphate.

Calcium ions play two roles in this catalysis mechanism. Ca^{2+} is nessessary to maintain an active site and facilitates the removal of diethyl phosphate from the active site. This is a reason to avoid the use of EDTA plasma in paraoxonase enzyme study (La Du 1989, Mackness 1997c).

It is undoubtedly true that PON-1 is important in metabolizing certain xenobiotics which are not found in nature such as organophosphate pesticides. Organophosphate pesticides are widely used in agriculture as the relatively non-toxic sulfur (thion) derivatives which are activated in vivo by cytochrome P-450 dependent microsomal monooxygenase to highly toxic oxygen (oxon) analogue by a process known as oxidative desulfuration (Mackness 1998, Akgur 2003).

The protection against organophosphate pesticide will depend not only on the level of enzyme activity in blood or tissues but also on the particular isoenzyme present as well. There are two types of isoenzyme A and B-isoenzyme which provided the PON1 activity. Polymorphism has been shown to be an amino acid substitution at position 192. The A-isoenzyme, low activity toward paraoxoanse, has glutamine (A) at position 192 while B-isoenzyme, high activity paraoxonase, has arginine.(B) B-isoenzyme is several times more efficient that A-isoenzyme in hydrolyzing paraoxon, but most organophosphates are hydrolyzed appreciably better by A-isoenzyme than B-isoenzyme as shown in Table 5. However, both levels of A and B isoenzyme must be taken into consideration in evaluating the protective role of PON1 against xenotoxic compounds, especially organophosphate pesticide (Mackness 1998, Furlong 2000).

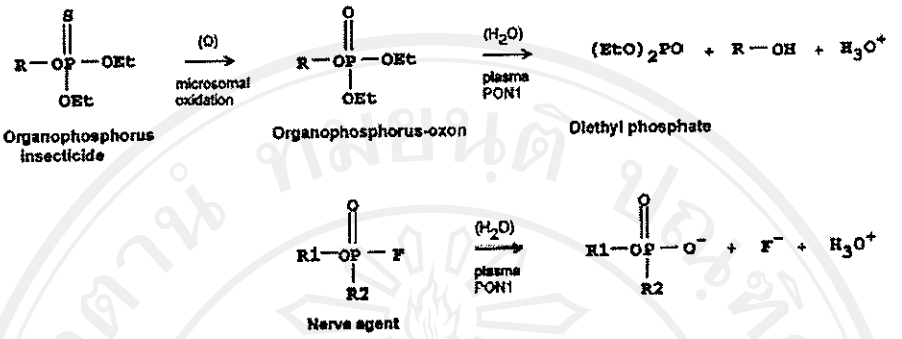


Fig. 1. The cytochrome P450/PON1 pathway for the bioactivation and subsequent detoxication of several organophosphorus insecticides and the nerve agents soman and sarin.

Figure 9 Paraoxonase pathway for detoxicfication of organophosphate insecticide (Akur 2003)

Table 5 The PON1 substrate activity polymorphism (Mackness 1998)

Isoenzyme	Specific substrate
B isoenzyme more active with	Paraoxonase, Methylparaoxon, Armin Chlorthion oxon, EPN oxon
Similar activity of alloenzymes	Phenyl acetate, Chlorpyrifos oxon 2-Naphthyl acetate
A isoenzyme more active with	Diazoxon, Sarin, Soman, Phospholipid

More over, there is another genetic polymorphism of PON-1 gene is codon 55 where has an amino acid substition at position 55, Leucine (L) and Methionine (M). There is a report, studied in 300 healthy people in the UK and found that position 55 modulate paraoxonase inpendently of the 192 polymorphism (Mackness 1997).

Thus individuals homozygous for the AA/MM polymorphisms have lowest activity toward paraoxon and BB/LL have highest activity. It has been suggested that genotyping for 192 polymorphism may provide a basis for determining a person's susceptibitiy to OP poisoning. However, both type of genetic polymorphism might be necessary to identify the persons most at risk of OP poisoning. This finding is potentially valuable for agricultural workers who may be at risk of OP poisoning, which results in a large number of deaths annually (Costa 1995).

The PON-192 genetic polymorphisms may have more effect on the paraoxonase concentration. A study in a healthy population found that homozygous BB have higher paraoxonase than homozygous AA, while heterzygous AB have intermediate level. Another study of PON-55 genetic polymophisms in non-insulin-dependent diabetes mellitus (NIDDM) patients showed that polymorphism on position 55 affected the paraoxonase, but had no effect with the healthy population. Many studies have investigated genetic polymorphism and its relationship to many diseases and health conditions. However, ethnic population is the factor most affecting PON1 genetic polymorphism. Table 6 and 7 showed variability of PON1 gene both on position 55 and 192 in different ethnic population (Allebrandt, 2002).

Table 6 PON1 codon 192 genotype distribution in healthy population.

Study	Population	N	QQ	QR	RR
Helbecqua et al., 2004	French	242	147 (61%)	86(35%)	9(4%)
Ferre et al, 2002	Spainish	430	211 (49%)	180 (42%)	39 (9%)
Sukru et al., 1999	Turkish	381	187 (49%)	153 (40%)	41 (11%)
Allebrandt et al., 2002	Afro-Brazilians	70	15 (21.4%)	36 (51.4%)	19 (27.2%)
Allebrandt et al., 2002	Euro-Brazilians	101	49 (48.5%)	42 (41.6%)	10 (9.9%)
Pati et al., 1998	Indians	80	60 (75%)	12 (15%)	8 (10%)
Suehiro et al., 1996	Japanese	252	34 (13%)	124 (49%)	94 (37%)
Ko et.al, 1998	Taiwanese	218	30 (13.8%)	96 (44.0%)	92 (42.2%)

Table 7 PON1 codon 55 genotype distribution in healthy population.

Study	Population	N	LL	LM	MM
Ferre et al, 2002	Spainish	430	164 (38.1%)	198 (46.0%)	68 (15.9%)
Sukru et al., 1999	Turkish	381	200 (52.5%)	147 (38.6%)	34 (8.9%)
Allebrandt et al., 2002	Afro-Brazilians	70	33 (47.1%)	34 (48.6%)	3 (4.3%)
Allebrandt et al., 2002	Euro-Brazilians	101	33 (32.7%)	57 (56.4%)	11 (10.9%)
Sanghera et al., 1998	Indians	183	119 (65.0%)	54 (29.5%)	10 (5.5%)
Sanghera et al., 1998	Chinese	181	168 (92.8%)	13 (7.2%)	0 (0.0%)

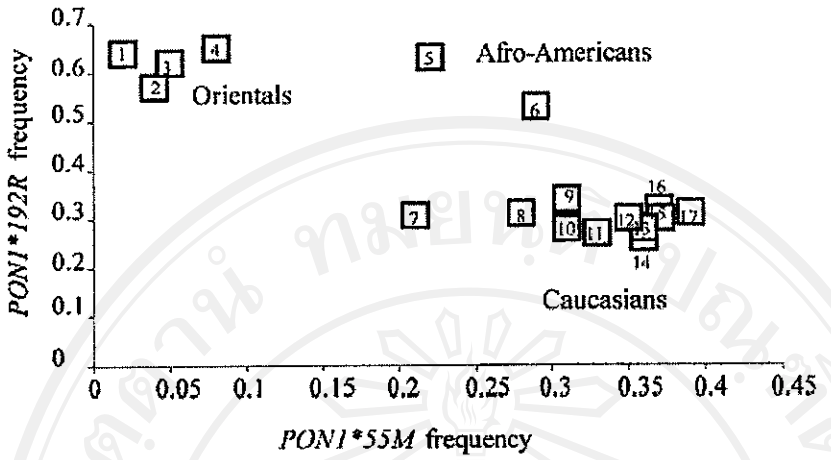


Figure 10 Distribution of M and R frequency in different ethnic populations

Abbreviation	Country	Number (n)	Study (reference)
1	Canada	46	Chan et al., 2000
2	China	142	Sanghera et al., 1998
3	Japan	134	Suehiro et al., 1997
4	Japan	431	Imai et al., 2000
5	Afro-Americans	46	Chan et al., 1999
6	Brazil	70	Allebrandt et al., 2002
7	India	175	Sanghera et al., 1998
8	Turkey	381	Aynacioglu et al., 1999
9	Canada	50	Chan et al., 2000
10	Russia	117	Akhmedova et al., 2001
11	Germany	971	Cascorbi et al., 1999
12	North America	317	Brophy et al., 2000
13	Germany	2784	Gardemann et al., 2000
14	England	279	Mackness et al., 1997
15	France	408	Blatter-Garin et al., 1997
16	The Netherlands	201	Leus et al., 1999
17	Brazil	101	Allebrandt et al., 2002

The data in Table 6 show the genotype distribution in different ethnic population. QQ (AA) homozygous of PON1 codon 192 was mostly found in Caucacian population, while RR (BB) homozygous was generally found in Oriental as Indian, Chinese and Japanese. It could be concluded that Q allele predominate in Cacasians and R allele is the allele that reaches the highest frequencies in orientals. The R (B) isoenzyme is more efficient in hydrolyzing paraoxon, but most of organophosphate compounds are better hydrolyzed by the Q than the R isoenzyme.

Table 7 shows the results of PON1 codon 55 polymorphism of Leucine (L) and Methionine (M) substitution. All of ethinic group were generally found LL and LM more than MM genotype. MM genotype was absent and low frequency in Chinese and Indians. Up to 15.9 percent of MM genotype was present in Caucacian. However, high linkage disequilibrium was detected both group and Asians, mainly due to the absence or very low frequency of the MR Haplotype (Allebrandt et al. 2002).

The genetic variants of PON1 have been referred to in association studies with disease and intoxication of organophosphate pesticides. Many studies suggested that PON1 variants determine differential susceptibility toward organophosphate compounds. Moreover, the ethnic composition may influence the population resistance to intoxication by pesticides and neurotoxic agents, since the R and Q alleles are the main determination of the paraoxonase activity toward organophosphorus compounds.