CHAPTER 2

LITERATURE REVIEW

2.1 Pesticide definition and history

Most pesticides are chemicals that are applied to control pests, weeds, or plant diseases in agricultural area. These chemicals may be extracted from plants or by chemical synthesis. FAO (1986a) defined a pesticide as any substance or mixture of substances intended for preventing, destroying, or controlling any pests, including vectors of human or animals diseases, unwanted species of plants or animals causing harm during, or otherwise interfering with, the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products, or animal foodstuffs, or which may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies (WHO, 1990).

Pesticides entered the organism's body and reached at the site of action and causing uncommonly of biochemical reaction and physiology function. If their entering rates are quicker than elimination, organisms will die because they accumulate until a toxic concentration is high enough to demolish target physiological function (Hassall, 1982).

Usage of pesticides was started in Greek and Roman Empires. They used fumigant from sulfur burning, arsenic, soda and olive oil for legume seed treatment. Next in China in 16th century, arsenic and extraction from tobacco were used as insecticides (Hassall, 1982; Hassall, 1990). In the 19th century, pyrethrum, soap, wash of tobacco, sulfur and lime had been used for insect and fungi control. The first systematic study in chemicals for crop protection work on arsenic compound was

occurred in 1867-1900 in Paris and USA. In 1913-1932, organomercury, tar oil, dinitro-orthocresol were used as pesticides. In the 2nd world war DDT found in Switzerland and OPs was developed in Germany. In 1945 CAs developed in Switzerland (WHO, 1990).

Recently, there are 4 major groups of pesticides classified by their chemical molecular structure and compositions. They are organophosphates (OPs), carbamates (CAs), organocholines (OCs) and pyrethroids. They affect on nervous system of insects and other organisms, but their utilities are different because of their properties and cost-benefit. OCs are able to kill widely type of pests but high persistence while OPs are lower persistence than OCs but they can kill only soft body insects. CAs are alternative choice but they are expensive, then they are used when OPs applications are failed. For pyrethroids, they are appropriate in killing household pests such as houseflies and mosquitoes (Hassall, 1990).

2.2 Organophosphate and carbamate Pesticides

Nowadays OPs and CAs are worldwide popular pesticides in agriculture including in Thailand (Rojee, 1995). Popular OPs include azinphos methyl, dichlorvos, parathion, methyl-parathion, fenithion, malathion, demeton methyl, dimethoate, monocrotophos and phosphamidon. Their properties are brownish liquid and smell like garlic. The majority of OPs are effective in the control of aphid and similar soft-bodies small insects, such as aphid, capsids, sucking pests, leftminers, sawflies, weevils, low persistence, ultra low spray (Hassall, 1982). Popular CAs are carbaryl, methomyl, propoxur, aldicarb and carbofuran and they are used to control

aphids, nematode, malaria and dengue and other insects which resist to OPs (WHO, 1990).

OPs were first developed in Germany. They were developed under the direction of Gerhard Schrader who engaged on the task of developing highly toxic nerve gas for potential use in warfare. Several OPs related to nerve gases, i.e., taban and sarin found to be effective insecticides. After ending of World War II many formulas of OPs were built and now there are 100,000 forms and 100 are marketed to use as pesticides (Hassall, 1982; Perring and Mellanby, 1977). They were developed for insecticidal activity, many are very toxic to vertebrates, and they can cause hazard to wildlife (Moriarty, 1983).

The first carbamate was physostigmine and was found in Calabar bean (*Physostigma benenosum*) in 1953 (Brown, 1978). It is a powerful anticholinesterase activity. Physostigmine is not very toxic to insects even though in vitro it is a powerful inhibitor of insect nerve cholinesterase. Its low in vivo toxicity is a consequence of its high degree of ionization at pH 7 and then difficult in penetrating insect cuticle and reaching the nervous system. The carbamate which attached N-methyl to form carbamate, make it lipophilic and penetrate to insect nerve system. They are more expensive than OPs and DDT about 8 time. However it is using when other pesticides are not effective (Hassall, 1982).

OPs and CAs have been introduced to replace OCs because they are biodegradable since 1945 (Perring and Mellanby, 1977). Most OCs were overtaken because the concern of persistence and polluting effect, such as DDT. Organocholine-free production therefore has become popular. However, a greater number OPs are continuously applied to crops during growing season (Hassall, 1982). Consequently,

OPs residues are significantly found in crops e.g. rice; and non-target organisms e.g. snail and fish (Tayaputch, 1998; Varca and Tejada, 1998).

OPs and CAs are highly toxic to human and non-target organisms (Lari *et al*, 1994). Because these compounds inhibit a whole range of cholinesterase enzymes, which are important regulators in the nerve system and an important one of these enzyme is acetylcholinesterase (AChE) (Moriarty, 1983). Consequently, in human, OPs in high dose are caused myocardial and conducting systems injuries. Primary symptoms are causing from irritation in gastrointestinal tract which patients are manifesting as intense nausea, vomiting and diarrhea (Rola and Pingali, 1993). After convulsions causing from hyperactivity of nerve impulse, bodies become weak and paralyzed, eventually die (Brown, 1978; Hayes and Laws, 1991).

The vertebrate nervous system is not completely different from insect nervous system. There is the mechanism which is occurred in both living-thing groups. It is the transmission of nerve impulses across nerve cell to other nerve cell or organs. When the nerve impulse reaches the end of axon, a very small gap, exists before the next nerve cell. This is called synapse. A similar gap exists between the last nerve cell and the effector cell (e.g. a muscle). A chemical transmitter carries the signal across the gap, and must be destroyed immediately after it has done its job or else the consequence would be the same as the second nerve cell (or the effector cell) are to receive a continuous impulse (Hassall, 1982). The area, which is called synapse, is sensitive area where OPs and CAs disturb until able to kill exposed organism. In synapse area has 2 main substances, neurotransmitter and enzyme regulator cholinesterase (ChE).

Neurotransmitters are chemical messengers that carry information from a transmitting neuron to a receiving cell, either another neuron or an effector cell (Campbell *et al*, 1997). For example, adrenalin and related catecholamines (noradrenalin, dopamine), which mediate nervous impulses by receptor attachment (Paasivirta, 1991) and an important one is acetylcholine (ACh) which is the most important neurotransmitter in the nervous system and at the neuromuscular junctions in the voluntary nervous system (Hassall, 1982; Paasivirta, 1991; Scaps *et al*, 1997).

Cholinesterase has two main members. One is true cholinesterase, or acetylcholinesterase (AChE), which occurs in vertebrate erythrocytes, the electric organs of eels and fishes and is the principal cholinesterase of the vertebrate nervous system and the insect central nervous system. Another member is called pseudocholinesterase (or serum or plasma cholinesterase) and occurs primarily in invertebrate blood plasma and to some extent in brain and other tissues. It has no known biological function and can be totally inhibited without showing toxic consequences (Perry *et al*, 1998).

Acetylcholine (ACh) is the natural substrate for AChE because AChE has a receptor for react with ACh. The receptors are active structure units (small area) of cell membrane protein, enzyme, nucleic acid, or other biomacromolecule. The receptor is like a lock, and a small transmitter molecule of a life process is like a key that fits perfectly into that lock. If a molecule of a foreign (Xenobiotic) chemical has a similar structure, size, and polarity, it may act as a "false key". Binding of a metabolism, execute a harmful metabolic process, or modify binding might cause acute or chronic poisoning or promote teratogenic or carcinogenic effects (Paasivirta, 1991).

The reaction between ACh and AChE is one of the principal known transmitters of impulses across synapses between adjacent nerve ending, and across neuromuscular junction. Nerve impulses stimulate the release of ACh, which is stored bound to protein in vesicles of a presynaptic neuron where a bioelectric current impulse liberates it to an active form. Next, ACh travels though the synapsis (the gap between nerve endings or ganglia) to irritate receptors on a postsynaptic membrane, and the nerve impulse proceeds (Paasivirta, 1991). The ACh is normally broken down rapidly by hydrolysis and catalyzed by the enzyme AChE (Moriarty, 1983). In order to restore the sensitivity of the postsynaptic membrane, the chemical transmitter must be eliminated. At cholinergic junctions this is accomplished by the enzyme cholinesterase which breaks down acetylcholine into its inactive compounds, choline (Ch) and acetic acid (Aa)(eq 2.1). At AChE receptor has hydoxyl group where is locking ACh molecule and splitting out Ch and released Aa (Moriarty, 1983). Another enzyme, acetyl coenzyme A (ACEA), joins Aa and Ch back to vesicle-bound ACh (Fig 2.1) (Paasivirta, 1991; Perry *et al*, 1998).

Acetylcholine + AChE → acetic acid + choline **eq 2.1**

The primary site of action of OPs is the enzyme (acetyl) cholinesterase because OPs have some structure similarity to the natural substrate, acetylcholine which is present in the nervous system (Xu and Bull, 1994). They are neurotoxic substances that effects by inhibition the enzyme AChE, thereby prolonging the residence time of acetylcholine at cholinergic synapses and producing hyperexcitation of cholinergic pathways (Roush and Tabashnik, 1990). This phenomenon may be

called inhibition of AChE which mean that ACh persists much longer, normal nerve function are grossly disturbed, and a sufficiently severe disturbance ends in death. The lethal lesion disturbs impulse transmission across synapses and neuromuscular junctions, many physiological processes are disturbed in consequence, and death, invertebrates at least, usually results from paralysis of the respiration system (Moriarty, 1983; Scaps *et al*, 1997).

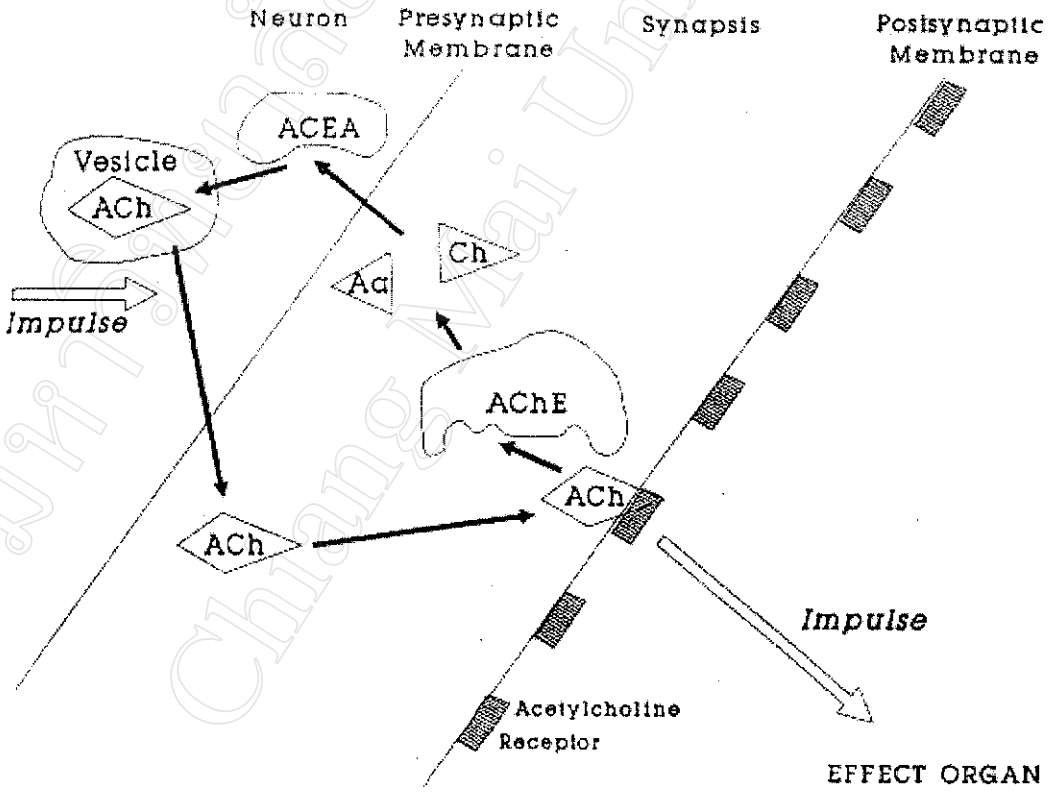

Fig 2.1 Cholinergic nerve impulse transmission in synapsis, nerve endings or ganglia (Paasivirta, 1991)

By mechanism, the enzyme-mediated hydrolysis of AChE depends on the attack by a hydroxyl group to a serine molecule in the enzyme. This hydroxyl group reacts which ACh to liberate choline, and the acetylated enzyme then resets with to regenerate the enzyme and to release acetic acid. This hydroxyl group in AChE is also the site of action for OPs. In general terms, the organophosphorus molecule become attached to the serine group of enzyme (phosphorylation), in much the same way as does ACh (Moriarty, 1983; Brown, 1978).

After they are applied to control pests in agricultural areas. Their side effect on human and the environment are of high concern. There was case study that shows inhibition of blood cholinesterase by OPs and CAs in workers exposed to these compounds. Acute clinical poisoning appears when the cholinesterase activity is inhibited by 50% or more and 30% inhibition has been proposed as a "hazard level". Cumulative cholinesterase inhibition can occur following exposure no clearly symptom is shown until cholinesterase activity is depressed lower the threshold (WHO, 1990).

In living-things there are detoxification mechanism that tried to eliminate OPs and CAs. The basic self-recovery is hydrolyzing with water (H_2O) to break OPs and CAs out of AChE active site (esteric site). This mechanism easier occurred with CAs than OPs molecules, because bonding between CAs and AChE are not strong and only 30 – 40 minute, half of CAs molecules are able to be hydrolyze out of AChE. But bondings between OPs and AChE are stronger (Kallander *et al*, 1996). Then there are mechanisms that able to eliminate OPs e.g. parathion metabolism which changes parathion to the last lower toxicity product (Fig 2.2). This mechanism requires many enzymes such as microsomal oxidase, phosphatase, GSH S-transferase and energy

NADPH (Fig 2.3) for example. But this mechanism produces more toxic intermediate product paraoxon from parathion (Fig 2.3).

The change of parathion to paraoxon (Fig 2.3) is oxidative desulphuration; substituted sulphur (S) with oxygen atom (O) that occurred in many thiophosphate insecticides. The new oxidative form is a better cholinesterase inhibitor than the original substance and in toxicity test there are found that paraoxon is higher toxicity than parathion for about 15 times (Hassall, 1990; Hayes and Laws, 1991).

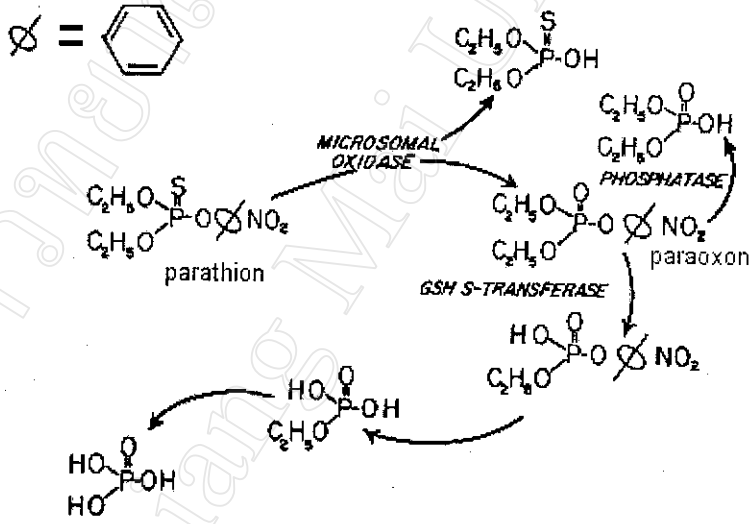

Fig 2.2 Parathion metabolism in mammals and insects (Matsumura, 1972)

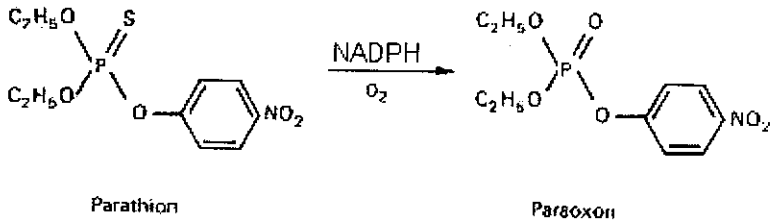

Fig 2.3 Reaction of parathion changing to paraoxon in parathion metabolism (Hassall, 1990)

2.3 Measurement of ChE activity

In 1956, Bonting and Featerstone introduced method for determination of cholinesterase to detect effect of OPs and CAs in living-things, but this assay not suitable to measure enzyme in small-scale (Ellman, 1961). Until 1961, Ellman method "a rapid paper showing method for studying acetylcholinesterase activity in tissue extracts, cell suspensions and blood by photometric method" was published. This method enables to measure enzyme activity when very low volume of samples (i.e. 10 µl sample of blood) available. Since then, his method has become worldwide acceptable. At that time, he tested this technique in human erythrocytes and some organs of the rat. Until recent years, his method was applied to detect impact of OPs in human blood (Seto *et al*, 1997). On the other hand, his method was popularly modified and applied for assessing these pesticides' risk by using other organisms in the environment (Scaps *et al*, 1997; Stanley, 1993; Ibrahim *et al*, 1998). Now WHO accept ChE measurement to monitor residues and the impact of OPs (WHO, 1990).

Monitoring AChE activity in wildlife population has been proposed as a general means for detecting environmental contamination from OPs and CAs, particularly since many of these chemicals have relatively short half-life in the environments i.e. parathion (14 days) carbaryl (10 days). Then AChE activity monitoring is commonly purposed to detect sublethal responses to OPs' exposure to the biochemical level before physiological and population effect occurred and it used as a parameter for testing water for the present of OPs and CAs. An adequate biomonitoring should show a significant AChE reduction in low and sublethal concentration proportional to ambient concentrations (Kuhn and Streit, 1994).

Ellman method to measure ChE activity is more easily and convenient than the residuals of the pesticides themselves that many of them break down rapidly in the body. Nevertheless, to prove which of these agents was existing, chemical analysis would be necessary. So the inhibition of AChE is a valuable biomarker to investigate agricultural land or areas likely to be affected by run-off from these areas, but would not be so useful in investigations outside these areas (Walker, 1996). Making use his method enable to indicate the impact and shows an early warning sign of the environmental degradation from heavy use of pesticides in agricultural fields.

As mentioned earlier Ellman method is able to measure enzyme activity in small quantity of samples (i.e. 10 µl sample of blood). He applied acetyl thiocholine (AChI) as substrate which an analog of true substrate with AChE. The essay measures the rate of production of thiocholine as acetylthiocholine is hydrolyzed. Thiocholine continuously reacts with 5,5-dithiobis-2-nitrobenzoate ion to produce the yellow anion of 5-thio-2-nitrobenzoic acid (show in eq 2.2). The rate of color production is measured at 412 nm (Ellman *et al*, 1961; Tor *et al*, 1994). This technique is also applied to detect the effect of other inhibitors to ChE. The ChE activity is calculated from Ellman equation, which showed in eq 2.3. This equation was based on the rate of DTNB degradation, If it degrade for 7 X 10^{-5} mol/l in 1 minute the absorbance will change for 1 unit.

acetylthiocholine + enzyme (AChE) → thiocholine + acetate thiocholine + dithio–bisnitrobenzoate + 5 thio-2-nitrobenzoic acid (yellow color) at 412 nm **eq 2.2**

Equation 2.3 (eq 2.3) Ellman's ChE activity (1961)

$$R = \frac{\Delta A}{1.36(10^4)} \times \frac{1}{(400/3120)C_0} \qquad \text{eq 2.3}$$

Where : R = Rate, in moles substrate hydrolyzed per min per g of tissue

ΔA = Change in absorbance per min

C_o = protein concentration (mg/ml)

Advantage of Ellman (1961) method was showed as following.

A. It is dependent on changes in the visible region of the spectrum, so that unusual changes in the absorbance can be checked immediately, e.g. appearance of turbidity spills on the photocell windows, etc.

B. Since the extinction coefficient of the nitrobenzoate ion is 13,600 as against 5140 for 5140 acetylthiocloline choride, there is at least a 2.5 fold potential increasing in sensitivity available.

C. Measurements of the appearance of products are usually more sensitive than disappearance of substrate.

D. Homogenates of tissue do not required any special handing, i.e. precipitation of protein before reading, etc.

E. The reagents required are commercial available.

Recently Ellman method is slightly modified such as, applied acetylthiocholine iodide (AChI) substituted acetylthiocholine choride has been widely

used as a model substrate for monitoring AChE activity in the presence and absence of insecticides. Furthermore, application of the kinetic microplate readers' technique allows the rapid and accurate measurement of AChE activities. The AChE is typically prepared by homogenizing the heads of insects in a buffer solution. Occasionally the addition of a small amount of detergent, such as 1% triton X-100, enable to obtain optimum activity (Roush and Tabashnik, 1990; Kuhn and Streit, 1994; Xu and Bull, 1994). The reaction are stopped with eserine before measure with spectrophotometry (Xu and Bull, 1994; Seto *et al*, 1997). There are alternative methods that avoided calculation of enzyme kinetics e.g. method for determination of the rate constants and degree of inhibition that are more convenient for rapid technique (Roush and Tabashnik, 1990; Tejada *et al*, 1998).

2.4 Pesticides impact on population and community

The population changed caused from pollution or stress in one species will be affect on others organisms or cause community change (Greaves *et al*, 1988; Able, 1989). Then with this observation, there were researches that had hypotheses to deduce the changing of non-target community is caused from impact of pesticides used. For examples, the studies belong to Josefo, 1994; Phalaraksh, 1995; Joshi, 1995. These investigations were designed to compare the aquatic macroinvertebrate community between pesticides contaminated against uncontaminated streams. In 1994, Josefo revealed the impact from highland agriculture on the Nong Hoi watershed, Mae Rim District. He found 38 time increasing of aquatic macroinvertebrate drift-out of their habitats in four hours after pesticides spraying. Diptera such as Chironimidae and Simulidae were the most group that drift out from

habitat. Phalaraksh (1995) found the diversity, evenness indices and community density of macroinvertebrates from surber sampler in contaminated Huai Nong Hoi stream (Mae Rim district) was statistical significantly lower than the control stream at Huai Chang Khian stream, Muang Chiang Mai district. Another investigation conducted by Joshi (1995) in the Mae Soi watershed, Chomthong District, where heavy pesticide spraying on cabbage plots, and again significantly impact on aquatic macroinvertebrate community by pesticides use.

In the other hand, some experiments were set to clearly explain the mechanism of pesticides effect to population before impact on community change. These experiments, for example, Kence and Jdeidi (1997) studied parathion effect on house fly larva population. They found many mechanisms that cause population change such as; emerging rate remain around 25% in 450 – 1000 eggs per 100 g of food and their body weight were remain only half. After that the rate of development was reduced from 12-13 to 11 days which was cause unfertile mature. Next step, there were some experiments were developed from laboratory to field condition which contain of other organism in system e.g. Hardensen *et al*, (1999) tested the impact of carbaryl in development and population of emerge damselflies *Xanthocnemis zealandica* (Odonata: Zygoptera) under field condition (water reservoir having natural condition). They found that carbaryl 100 ppb reduced emergence success at 10 and 1 ppb carbaryl added and control caused damselflies emerge in 5 month but at 100 ppb remain only 2 month. They found that mature damselflies population in 100 ppb carbaryl condition was significantly lower than that of 10 and 1 ppb and the control (0 ppb). They gave interesting recommendation about population change at not too high contamination of carbaryl (10 ppb) that might cause lethal or sublethal effect to

damselflies. Moreover the pesticide was able to kill their enemies, pathogens and parasites of this organism which support them to emerge successfully.

2.5 Environmental risk assessment

Numbers of investigations on the impact on the aquatic ecosystem have been more focused on. Therefore, the pesticides sprayed were usually drained or accidental spilled, and finally contaminated the nearby land and water resources. For examples, Kuhn and Streit (1994) studied the affects of organophosphates to acetylcholinesterase in gammarus. Ibrahim *et al* (1998) interested in the effects of organophosphorus, carbamate, pyrethroid and organocholine pesticides, and a heavy metal on survival and cholinesterase activity of of *Chironomus riparius* Meigen. These researches aimed at finding out suitable biomarker to reflex the dose response to pesticides contaminated in their habitat. These researches suggested that there were biological impairment from pesticides used in non-target organisms which indicate the environmental impact. The organisms with biological impairment were bioindicator.

Bioindicator relate with survivorship of organisms in stress environment. Its definition is organism which occurrence or non-occurrence of which or its specific reaction (e.g. acute mortality, population reduction, formation of necroses, accumulation of particular substances, physiological changes) indicate specific local environmental conditions (Nagel, 1995). Another definition is a related group or community of organism whose occurrence or an easily observed behavioral trail of which can be so closely correlated with certain environmental conditions that it can be utilized as a pointer or quantitative test given by Ellenberg *et al* (1991). In the

biological monitoring of pollution (like pesticide applications), indicator organisms are susceptible to the pollutants. While sentinel organisms can be used to assess the scale and distribution of the pollutant by identifying the amount of the residue of the pollutant in their tissues (Nagel, 1995). Consequently nowadays, there are 3 sub-divisions of bioindicator to identify the environmental impact. They are biosensor, bioaccumulator and biomarker (Fig 2.4).

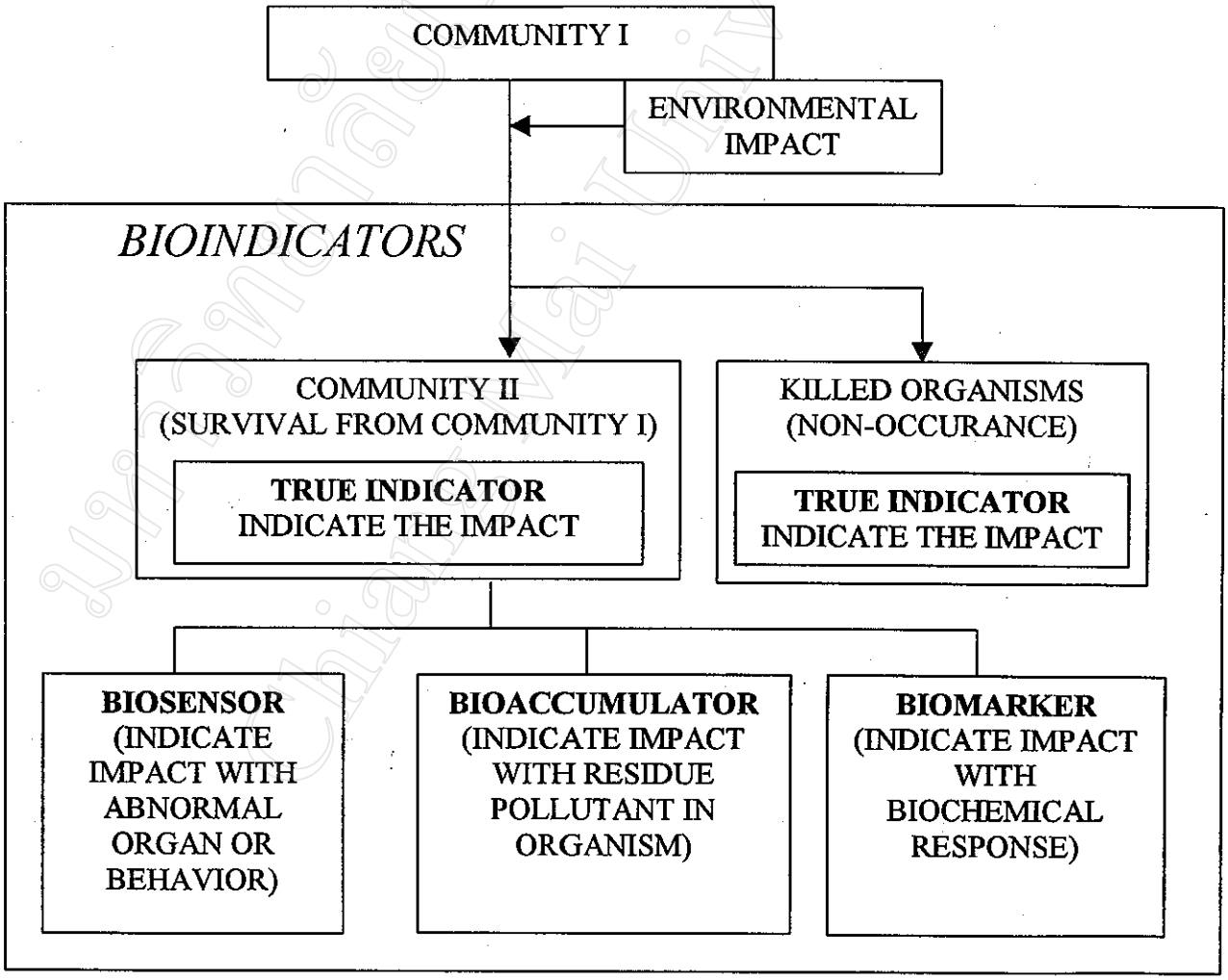

Fig 2.4 Flow chart shows application of bioindicators to indicate environmental impact (Modified from: Hellawell (1986), Calow (1993), Nagel (1995))

Biosensor is an indicator that continuously exists in a polluted environment but suffer physiological stress, which is revealed in diminished rate of growth or impaired reproductive capacity or modified behavior (Hellawell, 1986). These indicators indicate impact with their abnormal organ or physiology changing causing from pollution. For example of biosensor using, Jeffrey and Madden, 1991 advised to apply molecular biology and cytometric technique of microorganism to detect pathogen contamination from polluted environment.

Bioaccumulator is organism which accumulates toxic substances in its tissue in a way so as reflect environmental levels of those substances or the extent to which the organism has been exposed to them (Hellawell, 1986). Birds of pray, top carnivore, are always used to reveal contamination of lead (Pb) which they uptake from their preys and the residue magnify along the food web (Persoone *et al*, 1990). Tissues of fish or mussel are usually used for detection of DDT and other OCs analogs (Tabucanon and Boonyatumanond, 1998).

Biomarker is a biochemical or cellular response that can be related quantitatively to the extent of exposure to a chemical (or class of chemicals) and can be used as a bioassay of the presence and effects of significant pollutants (Calow, 1993). Impact is identified with changing biochemical response e.g. cholinesterase activity reduction causing from OPs and CAs (Kuhn and Streit, 1994; Scaps *et al*, 1997; Ibrahim, *et al*, 1998). Most appropriate biomarkers have characteristics shown as followings (Moriarty, 1983).

1. Widely distribution and abundant in studied site, although they are frequently sampled from the habitat but the are remaining still enough to stabilize the community

2. Easy to identify and collect
3. Big enough to enumerate
4. Sedentary at most stage of their life-cycle
5. Easy to identify age
6. More tolerance, contain level of pollutant that are neither too low as to make chemical analysis difficult nor too high to give ill or kill individuals
7. Well-known species with more detail of their autecology
8. Initial of food chain better than the end
9. Play important role such as being economical organisms

The indicator organisms must be selected from self-experiment or acceptable literature before using bioindicator. These experiments concerning bioassay which organisms are used as test subjects and study their response to the applied stimuli. For example, pesticide bioassay is experiment done with appropriate amount of pesticide to get the desired response (e.g., die, become sterile, or at the very least, suffer horribly) (Robertson and Preisler, 1992). In fact, bioassays are experiments interesting in the ability to kill of pesticides or biological responses of tested organisms to other chemical substances. Currently, bioassay is the main experiment in environmental concern because it deeply related with bioindicator application.

2.6 ChE activity as biomarker of exposure to OPs and CAs in aquatic ecosystem

An appropriate organism as a biomarker in aquatic ecosystem is fish (Moriarty, 1983). From bioassay experiment, the ChE activity test was done in fish, from many *ex situ* tests showed the enzyme inhibition of 40-80% which caused lethal (Stanley, 1993). However, the test in the true environment (*in situ*) hardly operated because fish are able to detoxify of some OPs and able to escape from exposure to pesticides (Kuhn and Streit, 1994). Then using fish as biomarker is successful in only heavily aquatic ecosystem.

For exclusion of escaping species, Scaps *et al* (1997) studied polychete *Nereis diversicolor* (ragworm) as a biomarker to detect OPs' impact in estuarine and salinity ecosystem. They recommended this species could be used for monitoring the effects of OPs in estuarine ecosystem because they burrow and live under substrate. Therefore in their pilot test, they found the significant response of AChE in ragworm. AChE activity was reduced more than 50% in 7 days when this polychete lived in water that contaminated by 10^{-6} M of malathion, parathion-ethyl and phosalone (all of them are OPs).

In fresh water ecosystem, chironomids and gammarus are recommended as the bioindicator because there are continuously studied worldwide. Ibrahim *et al*, (1998) found *Chironomus riparius* were killed for half of the population in 24 hours by OPs' concentration around 63.8 µg/l. They were killed when their AChE were reduced for 20% (equal with 20% inhibition). The same species was studied by Kallander *et al*, (1996), they found the recovery of AChE activity in *Chironomus riparius* with pulse; exposed 1 hour and unexposed 1 hour with 26 µg/l of aldicarb and 40 µg/l of carbaryl while continuously exposed to the same dose of carbaryl did not found. But in

organophosphate contamination (carbofuran 2.5 µg/l, malathion 32 µg/l, parathion 55 µg/l and propoxur 25 µg/l) did not found recovery either pulse or continuously exposure.

Gammarus pulex was reportedly very sensitive to OPs, at 1 µg/l of parathion-methyl or fenithion it's AChE activity was reduced for 61-65%. Other gammarids; *Gammarus fossarum* and *G. tigrinus*, showed the results not difference with *G. pulex*. Kuhn and Streit (1994) selected them to test sublethal effects of OPs by measuring AChE activity. They gave the idea of this experiment that sublethal might lead to lethal in the environment by and cause behavioral change e.g. easy to drift, easy to predated because of escaping effort was reduced, reproduction behavioral change and etc.

2.7 Existing information of study site

Stuetz reported his survey of the use of pesticides and their impact on the health among the Hmong villagers in Mae Sa Mai village, Mae Sa Noi watershed, Mae Rim district, Chiang Mai. He reported the mostly used pesticides were OPs (parathion and mevinphos were most popular used) and CAs (methomyl and carbaryl), details of them were shown in table A9 (appendix). They have been heavily used in cabbage and lychee fields in Mae Sa Noi watershed, Mae Rim district (Stuetz, 1999). The OPs and CAs have tendency to increasing used because the resistant ability of insects and monocrop planting especially on the highland fields particularly among the hill tribe farmers where good agricultural practice (GAP) hardly in practice. Hence, the environmental toxicology assessment of OPs and CAs using macroinvertebrate might be the first of its kinds in this part of Thailand. In the same

time chironomids were used as biomarker to detect molecular impact of OPs and CAs used. Chironomids were selected because of their were common and abundant in all study sites while the method to detection and test condition was available. Moreover, they are dipteran (fly, midge, mosquitoes) that having high AChE activity; (65-90%) of the AChE activity in the head, more than hemipterans or coleopterans (XU and Bull, 1994).

Chironomids are the name of living-organisms in family Chironomidae, Order Diptera, Class Insecta, Phylum Arthropoda. In larva stage, they are slender, commonly cylindrical and slightly curved body. They're long for 2-20 mm or occasional larger. The body has a pair of prothoracic prolegs and a pair of terminal prolegs. Their last segment usually has a short dorsal pair of tubercles or projections, each with a variable tuft of hairs (MaCafferty, 1981).