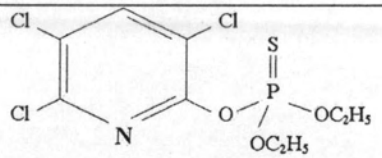
# CHAPTER II

# LITERATURE REVIEW

## 2.1 Chlorpyrifos

Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] is an organophosphorus pesticide (insecticide, acaricide, miticide, and mosquitocide) used for agricultural and non agricultural pest control. Technical chlopyrifos is a white crystalline solid with a melting point of 41-42 °C. In neutral and acidic solution, chlorpyrifos is stable; however, the stability decreases with the increasing pH. Chlorpyrifos is practically insoluble in water, but it is soluble in most organic solvents (i.e., acetone, xylene, and methylene chloride). Based on its low vapor pressure of $1.87 \times 10^{-5}$ mmHg at 20°C, chlorpyrifos is not particularly volatile (US EPA, 2002a). The other important physical and chemical properties of chlorpyrifos are shown in Table 2.1.

**Table 2.1** Physical and chemical properties of chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate]

| **Physical and Chemical Properties** | |
|---|---|
| Molecular Formula | $C_9H_{11}C_{13}NO_3PS$ |
| Structural Formula |  |
| Color | White/colorless crystalline solid |
| Odour | Odourless |
| Molecular Weight (g/mol) | 350.57 |
| Density ($g/cm^3$) | 1.40 |
| Melting Point (°C) | 41-42 |
| Vapor Pressure (mmHg) | $1.87 \times 10^{-5}$ |
| Water Solubility (mg/l) | 2 (25 °C) |
| Henry's Constant (atm $m^3$/mol) | $1.23 \times 10^{-5}$ (25 °C) |
| Log Kow | 5.11 |
| Log Koc | 3.73 |

| **Physical and Chemical Properties** | |
|---|---|
| Hydrolysis Half-Life (day) :<br>pH 5, 7, 9 | 72.8, 72.1, 29.4 |

Source: Ladaa et al., 1998

Chlorpyrifos has been first registered in the U.S. since 1965 for control of foliage and soil-borne insect pests on a variety of food and feed crops. The pesticide was one of the most widely used organophosphorus insecticides in this country and was one of the major insecticides used in residential settings until 2000 when most of residential uses were cancelled. Presently, registered chlorpyrifos is allowed for pest control in food and feed crops, golf courses, nursery and greenhouse, non-structural wood treatments, and public health (as adult mosquitocide). All use of products for structural termite control has been prohibited after December 31, 2005, unless acceptable data demonstrate that risks from these exposures are not of concern (US EPA, 2002a).

In Thailand, hundreds of formulated compounds containing chlorpyrifos as active ingredient have been imported and registered to office of agricultural regulation for agricultural purposes for more than 10 years (Toxic Substance Information Center 2002). Currently, there types of chlorpyrifos are allowed to register to Department of Agriculture, including 5% chlorpyrifos pellet, 20% and 40% emulsified concentration (Department of Agriculture, 2007). During 2003 to 2005, the average import value of chlorpyrifos was 140 tons or 800 million baht (The customs department, 2006).

The pesticide has been recommended for the control of Cotton bollworm, Subterranean ant, aphid, leafhopper, sweet potato weevil, angoumois grain moth, rice weevil, corn weevil, red flour beetle, siamese grain beetle, rice hispa, and pin-hole borer in soybean, peanut, sweet potato, rice grain, kapok, oil palm coconut tree, and banana tree. There is no recommendation for pest control in vegetables and fruits, however, chlorpyrifos residues were found in okra, lemon grass, and mangoes exported to Japan during 2003-2004 (Department of Agriculture, 2007).

Moreover, chlorpyrifos has been used for household, public health, and activities related to recreations purposes, e.g. domestic termite and insect pests control at golf course (Community Pharmacy Association, 2004).

According to the widely uses, environmental contamination of chloryrifos in Thailand has been reported for more than 10 years. Chlorpyrifos was found to contaminate water (0.78-1.5 µg/l) in the golf course adjacent to Nong Klang Dong Reservoir, the public reservoir, in Chon Buri Province during rainy season 1992 (Nayavon, 1996). The pesticide was reported to contaminate surface water (0.02 µg/l) in agricultural areas in Chiangmai, northern Thailand, one of the most well known areas for tangerine and other vegetation production in rainy season 2002 (Ciglasch 2003).

Although the environmental contamination and toxic effects of chlorpyrifos have been reported, there is no maximum acceptable value of this pesticide and all other organophosphorus pesticides for surface water of Thailand.

## 2.2 Toxicity of chlorpyrifos and molecular responses for the pesticide exposure

### 2.2.1 Acute toxicity

The insecticidal action of chlorpyrifos is, like most organophosphorus compounds, due to the irreversible inhibition of acetylcholinesterase (AChE), resulting in the accumulation of the neurotransmitter, acetylcholine, at nerve endings. This results in excessive transmission of nerve impulses, which causes mortality in the target pest.

**Table 2.2** Qualitative description for categories of fish and aquatic invertebrate toxicity (from Zucker, 1985)

| **LC50 or EC50** | **Category description** |
|---|---|
| < 0.1 ppm | Very highly toxic |
| 0.1- 1 ppm | Highly toxic |
| >1 < 10 ppm | Moderately toxic |
| > 10 < 100 ppm | Slightly toxic |
| > 100 ppm | Practically non-toxic |

The acute toxicity studies of chlorpyrifos to non-target aquatic organisms have been performed mostly on adult fish and indicated the high toxicity (Table 2.2) of chlorpyrifos on most fish species. The studies on acute toxicity of this pesticide for fathead minnows, *Pimephules promelus*, sheephead minnows, *Cyprinodon variegates*, and Indian carp *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala* showed the 96 h $LC_{50}$ were in the range of 0.122-0.650 mg/l (ppm). However, very highly toxicity of chlorpyrifos was found in some atherinid fishes, including *Menidia menidia*, *M. peninsulae*, *M. beryllina*, and *Leuresthes tenuis*, of which the 96 h $LC_{50}$ were between 0.0013 and 0.0042 mg/l (Clark et al. 1985; Jarvinen et al. 1988; Tilak et al. 2004).

Only few investigations have been carried out in aquatic crustaceans and most of them were conducted on larval stages. The results showed very highly toxicity of chlorpyrifos in larvae of mysid shrimp (*Neomysis integer*) and grass shrimp (*Palaemonetes pugio*), of which the 96 h $LC_{50}$ of chlorpyrifos were 0.00013 and 0.00015 mg/l, respectively (Roast et al. 1999; Key and Fulton 2006). In contrast, chlorpyrifos was moderately toxic to *Artemia salina* and *Litopenaeus stylirostris* larvae, of which the 96 h $LC_{50}$ were 3.19 mg/l and 2.26 mg/l, respectively (Reyes et al. 2002; Varo et al. 2002).

### 2.2.2 Neurotoxicity

Chlorpyrifos is a nerve toxin due to its function serves as inhibitor of acetylcholinesterase (AChE). Inhibition of AChE is reported to be the most sensitive effect of organophosphorus compounds in tested animal species and humans, regardless of exposure duration (US EPA, 2002b).

Chlorpyrifos has been found to affect AChE activity in many groups of organisms. Inhibition of AChE activity in postmitotic sympathetic neurons of Holtzmann rats was found after exposed to 10 μM chlorpyrifos for 72 h (Howard et al., 2005). The inhibition was also found in amphibian larvae and juvenile fish. Dose dependent AChE inhibition in *Xenopus laevis* was found after exposure to 100 μg/l chlorpyrifos for 72 h (Colombo et al., 2005). For juvenile Chinook salmon (*Oncorhynchus shawytscha*) significant inhibition of AChE activity in both brain and muscle was found after 96 h of exposure (Wheelock et al., 2005; Eder et al., 2004). Dose and time dependent exposure of chlorpyrifos through the skin of earthworm

(*Eisenia foetida*) resulted in the inhibition of AChE activity (at 0.0158 µg/l for 12 h (Rao et al., 2003)

### 2.2.3 Genotoxicity

It has been reported that various agrochemical ingredients possess mutagenic properties inducing mutations, chromosomal alterations or DNA damage. Chloryrifos was also reported to be genotoxic and mutagenic substance because it caused strand breaks and alterations in DNA molecules of tested organisms. The damage of DNA by chlorpyrifos was believed to be due to phosphorylation of the pesticide to DNA strands (Wild, 1975). However, a few number of literature reports on the assessment of genotoxic effects of chlorpyrifos on non-target aquatic invertebrates were found.

DNA damage in Swiss albino mice leucocytes was found after the *in vitro* exposure to chlorpyrifos within 24 h (Rahman et al. 2002). The reductions in protein and DNA in shrimp larvae, *Litopenaeus stylirostris* was found after exposed to sublethal concentrations of chlorpyrifos for 4 days.

## 2.3 Biochemical responses for chlorpyrifos exposure

### 2.3.1 Induction of Cytochrome P450

Cytochromes P450 are a super family of monooxygenase enzymes. The major function of these enzymes is to introduce oxygen into, or remove hydrogen from, either endogenous or xenobiotic organic substrate. It usually results in increasing the hydrophilicity of the substrate and altering its pharmacological or physiological activity. The monooxygenation of xenobiotics is usually catalyzed by members of cytochrome P450 families 1-4 (James et al., 1996).

According to the function, the enzyme can be induced by various xenobiotic species at the transcriptional level. There have been reports about induction of cytochrome P450 by xenobiotics and usage of the enzymes as biomarker for both field study and laboratory experiment in tested organisms, e.g. *Carcinus aetuari*, *Daphnia pulex*, and *Carcinus maenas* (Fossi et al., 1998; David et al. 2003; Rewitz et al., 2003).

Chlorpyrifos has been reported to activate cytochrome P450 level in preweanling rats from oral exposure to sub-lethal dose (10 mg/kg) of the pesticide (Timchalk et al., 2006). Examination of cytochrome P450 induction in German Cockroach *Blattella germanica* exposed to 2.6 $\mu g/cm^2$ chlorpyrifos for 72 hr indicate the increase in total cytochrome P450 level (Scharf et al., 1998).

**2.3.2Glutathione-s-transferase**

Glutathione-s-transferase is a multi-component enzyme that utilize glutathione in reactions contributing to the transformation of a wide range of compounds, including carcinogens, therapeutic drugs, organophosphorus compound, and products of oxidative stress. The enzyme plays a key role in the detoxification of these substances and has an important role in protecting tissues from oxidative stress (Foumier *et al.* 1992; Hodge et al. 2000).

Glutathione-s-transferase activity has been evaluated as biomarker for organophosphorus pesticide exposure. The reports were found in Tasmanian lacewing, *Micromus tasmaniae* (Hodge et al. 2000) and sheep blow fly, *Lucilia cuprina,* (Wilson and Clark, 1996).

According to sub-lethal concentration exposure of chlorpyrifos, the whole body glutathione-s-transferase activity of amphipod, *Hyalella azteca*, Tasmanian lacewing, *Micromus tasmaniae*, and *Boophilus microplus* were reduced with the increasing of pesticide concentration (Steevens and Benson, 1999; Hodge et al., 2000; Vaz et al., 2004). The Inhibition of liver enzyme activity was found in rat administered by sub-lethal concentration of the pesticide (13.5 mg/kg body weight for 8 weeks) (Goel, et al. 2005).

**2.3.3 Beta glucuronidase**

Beta glucuronidase is enzyme widely distributed in tissues and cells of the animal body. Beta glucuronidase is thought to be loosely bound to egasyn, a carboxylesterase isoenzyme. Exposure to organophosphorus or carbamate pesticides followed by the cleavage of the egasyn–glucuronidase complex leads to a rapid increase of plasma beta glucuronidase activity (Hernández, 2004).

It has been reported that beta glucuronidase activity could be considered as a biomarker of organophosphate and carbamate pesticides exposure in tested organisms. Rat serum beta glucuronidase activity markedly increased up to 400-fold after administration by sub-lethal doses of paraoxon (Williams, 1969). The induction of enzyme activity by the increasing doses of parathion and malathion was found in normal and protein-deficient rats treated by sub-lethal doses of the pesticides (Chakravarty and Sreedhar, 1982; Bulusu and Chakravarty, 1984). Activities of plasma beta glucuronidase were associated with organophosphates and carbamates exposure in humans (Hernández, 2004).

For chlorpyrifos, beta glucuronidase activity was found to be a sensitive biomarker of acute chlorpyrifos exposure. After administrated by chlorpyrifos, rat plasma beta glucuronidase activity increased in approximately 100-fold higher than that of the control level (Fujigawa et al. 2005).

### 2.3.4 Heat shock protein 90

Heat shock protein 90 is a cellular chaperone protein required for the activation of several eukaryotic protein kinases (Memorial Sloan-Kettering Cancer Center, 2006). This protein is believed to assist cells to adapt or survive by a rapid but transient reprogramming of cellular metabolic activity to protect cells from further oxidative and thermal stresses in responsive tissues. The protein plays an important role as biomarker for stressors or unfavorable conditions due to the increased production of this protein after exposure to stressors (Bagchi, 1996).

Chlorpyrifos was found to enhanced synthesis of heat shock protein 90 at the transcriptional level in cultured neuroactive PC-12 cells of female rats after 24 h of exposure to 50 mM chlorpyrifos (Bagchi et al., 1996).

### 2.3.5 Biodegradation of chlorpyrifos

Biodegradation of chlorpyrifos is mediated by multiple detoxifying enzyme systems (monooxygenase, type A esterase, and type B esterase). Parent chlorpyrifos is monooxygenated by cytochrome P450 through (1) oxidative desulfuration to form chlorpyrifos-oxon, an active form of acetylcholinesterase inhibitor, or (2) dearylation to form 3, 5, 6-trichloro-2-pyridinol (TCP), an inactive form. In addition, type A and

type B esterase metabolism of chlorpyrifos-oxon also forms TCP through hydrolysis. Therefore, the balance between desulfuration and dearylation of the parent chlorpyrifos results in different levels of acetylcholinesterase inhibition because chlorpyrifos-oxon is a key substrate of esterase. Although esterase involves in the detoxification of the pesticide, difference reaction between enzyme and substrate is performed. Whereas type B esterases such as acetylcholinesterase, butyrylcholinesterase, and carboxylesterase are inhibited by the oxon, type A esterases (PON1) are not inhibited and hydrolyze the pesticide to the inactive form (Poet et al., 2003).

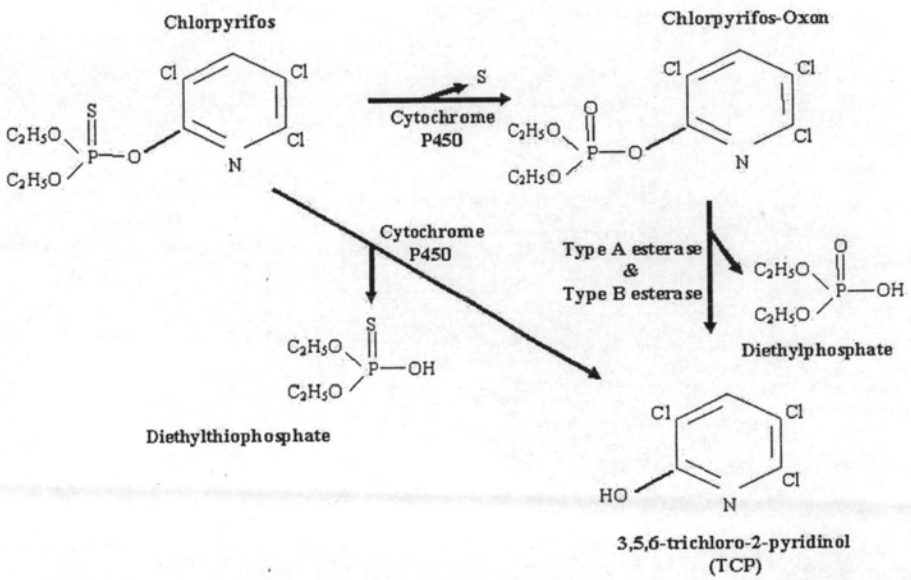



**Figure 2.1** Metabolic pathway for chlorpyrifos and their major metabolites chlorpyrifos-oxon, TCP , diethylphosphate and diethylthiophosphate, modified from Poet et al., 2003.

## 2.4 Application of biomarkers

### 2.4.1 Biomarkers

Biomarkers are defined as quantitative measures of changes in the biological system that respond to either (or both) exposure to, and/or doses of, xeonbiotic substances that lead to biological effects. The term biomarker is often used restrictedly to cellular, biochemical, molecular, or physiological changes that are

measured in cells, body fluids, tissues, or organs within an organism and are indicative of xenobiotic exposure and/or effect. (WHO, 1993; Hulka, et al. 1991).

Biomarkers can be divided into 3 groups:

1) **Biomarkers of exposure** provide early warning signals that are indicative to the exposure to a specific agent and the changes are not necessarily link to the harmful (toxic) effects in the target organism.

2) **Biomarkers of effect** are biological changes occurring in organisms and caused by contaminants.

3) **Biomarkers of susceptibility** indicate the inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance. (NRC, 1987; WHO, 1993)

A wide range of biomarkers had been developed and suggested for use in monitoring programs. Biomarker has been used both in vivo and in vitro for the evaluation of xenobiotic effects. One of the advantages of biomarkers is that it can indicate biological effects, while chemistry-based surveillance system cannot. It can determine the changes before a real damage has taken place. There is evidence that many biomarker responses are not directly associated with real harmful effects in the target organisms (Halander, 2003). In recent years, there has been considerable interest in the use of biomarkers for the early-warning systems. This involves knowledge of their biological function and it is necessary to identify possible interferences that can influence these responses in order to standardize the analytical procedure.

### 2.4.2 Use of biomarkers in field studies

The use of biomarkers for the determination of xenobiotic contamination has been increasingly adopted as part of the environmental monitoring program. Field study conducted at Vilaine River estuary, France, used the colorimetric method (Ellman et al., 1961) to measure acetylcholinesterase activity in copepods, *Tigriopus brevicornis* as biomarker for detecting the contamination of various neurotoxins, including triazines, amids, oxadiazoles, piperidines, morpholines, phenylamids, oxazolidine derivative, and methylparathion in the area. The inhibition levels of acetylcholinesterase activity from copepods in such area were 70-80% higher than

that of the reference site (Forget et al., 2003). Benzo(*a*)pyrene hydroxylase activity of *Carcinus aestaurii* was used as biomarker for polycyclic aromatic hydrocarbon (PAH) contaminated in Mediterranean lagoons. By using the colorimetric method of Kurelec et al. (Kurelec et al., 1977), benzo(*a*)pyrene hydroxylase activity from hepatopancreas of crab from Chioggia harbour, a high PAHs contaminated area, and Porto Marghera, one of the most important industrialized harbours in Italy, was 3.5 time higher than that of the other lower contamination areas (Fossi et al., 1998). Expression of Cytochrome P450 in *Daphnia pulex* was reported to be a sensitive biomarker of polyphenol rich area in the sub alpine temporary aquatic habitats in France. Using semi-quantitative RT-PCR followed by southern blot hybridization, the expression level of *CYP4C32* of animal inhabiting the polyphenol-poor area was 6 time higher than that from polyphenol-rich habitat (David et al., 2003).

## 2.5 Techniques used for examination of chlorpyrifos exposure and toxicity

### 2.5.1 Toxicity test for identification of acute toxicity

Toxicity test is needed in water pollution evaluation due to chemical and physical test alone are not sufficient to assess potential effects on aquatic organisms. To examine acute toxicity, which is a relatively short-term lethal or other effect, usually defined as occurring within 4 d for fish and macro invertebrate, toxicity test has to be done.

Toxicity test can be divided following the method of adding test solutions into 3 types:

1) **Static test** is the test in which solution and test organisms are placed in test chambers and kept for the duration of the test.

2) **Renewal test** is the test in which organisms are exposed to solutions of the same composition that are renewed periodically during the test period. This is accomplished by transferring test organisms or replacing test solution.

3) **Flow-through test** is the test in which solution is placed continuously in test chambers throughout the test duration.

To conduct short-term test, the technique can be static, renewal, or flow-through test. Exposure period for these tests are 48 h or 96 h. Static or renewal tests are less expensive to perform than flow-through tests. However, the flow-through test

is suit for high-BOD or COD system and for the test of unstable or volatile substances (APHA, AWWA, WEF, 1992).

**2.5.2 Colorimetric method for examination of AChE activity**

The method used in this study was base on Ellman 1961. The principle of this method is the measurement of the production rate of thiocholine as acetylthiocholine (an analog of the natural substrate, acetylcholine, of AChE) is hydrolyzed. This is accomplished by the continuous reaction of the thiol (thiocholine) with 5, 5-dithiobis-2-nitobenzoate ion to produce the yellow anion of 5-thio-2-nitro-benzoic acid as shown in Figure 2.1. The rate of color production is measured at 412 μm in a photometer. The activity of the enzyme is generally expressed as a rate: the quantity of substrate (in mole) which is broken down by a known amount of enzyme per unit of time. In this case, it is the amount of acetylthiocholine which is broken down by AChE per minute.

$$\text{Acetythiocholine} \xrightarrow{AChE} \text{thiocholine} + \text{acetate}$$

$$\text{thiocholine} + \text{dithiobisnitrobenzoate} \longrightarrow \text{Yellow color}$$

**Figure 2.2** The coupling reaction for AChE assay

**2.5.3 Single cell gel electrophoresis for examination of genotoxicity**

To measure DNA strand break, the single cell gel electrophoresis or comet assay is a rapid, sensitive and in expensive method. This method has advantages over other DNA damage methods, such as sister chromatid exchange, alkali elution, and micronucleus assay, because of its high sensitivity and the DNA strand breaks are determined in individual cells.

The technique is performed by dispersing and immobilizing cultured cells or isolated cells in an agarose gel coated on appropriate support media, such as microscope slides. The fixed cells are lysed with alkaline lysis solution to disperse

cell components, leave the immobilized DNA in the agarose. The DNA is denatured in an alkaline solution. Strand breaks in the denatured cellular DNA result in supercoil relaxation. The more breaks lead to the greater the degree of relaxation. The application of an electric field across the slides creates a motive force by the charged DNA may migrate through the surrounding agarose away from the immobilized nuclear DNA. The DNA in the fixed slides is stained with a fluorescent DNA-specific dye. DNA binding dyes which can be used for comet assay included ethidium bromide, propidium iodide, etc. Stained slides are examined using a fluorescent microscope. Optimal magnification will depend on the quantity of DNA in the cells being assessed for DNA damage. The migration of DNA away from the nucleus can be measured by eyes using an ocular micrometer or image analysis software to determine various parameter of the comet, i.e. tail length, percentage of DNA in tail, tail moment (Lee and Steinert, 2003).

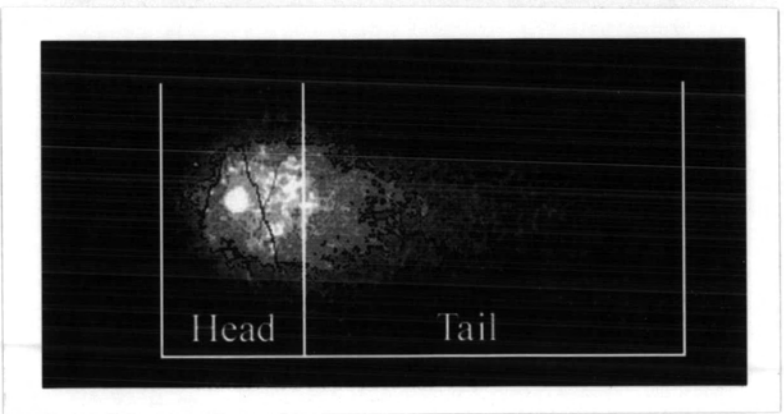



**Figure 2.3** Diagram of typical comet showing distribution of DNA in tail and head.

### 2.5.4 RT-PCR (Reverse Transcription PCR) and Semi-quantitative RT-PCR

Reverse transcription polymerase chain reaction (RT-PCR) is a technique for amplifying a defined piece of a ribonucleic acid (RNA) molecule. The RNA strand is firstly reverse transcribed into its complementary DNA (cDNA), followed by amplification of the obtained cDNA using polymerase chain reaction. RT-PCR differs from the conventioal PCR by cDNA is used as template rather than genomic DNA. The method is has been used to determination of gene expression in mRNA population (Kawasaki et al., 1990).

Semi-quantitative RT-PCR is a quantitative technique used to quantitate the relative amount of mRNA as cDNA from the starting samples. Target cDNA is separately or co-amplify with the internal control gene, such as beta actin, elongation factor 1 alpha, using the same template. Use of internal control gene is under the criterion that they are transcribed constantly and independently from the environmental stimuli.

**2.5.5 mRNA-Differential Display RT-PCR (mRNA-DD RT-PCR)**

This is one of various methods used to identify differences in gene expression. The method was introduced in 1992 and based on PCR amplification of random subsets of genes from different groups of RNA samples. The first step is to reverse-transcribe mRNA to cDNA using anchored primer(s), which is typically a polyT oligonucleotide with one or two additional bases. The primer anneals to the polyA tail of mRNA. Following the reverse transcription of first strand cDNA, segments of the cDNA transcripts are amplified using multiple PCR primer pairs. The forward PCR primer is an arbitrary primer, typically 10 bp in length, and coupled with the anchored primer used to synthesize cDNA. It has been estimated that PCR products obtained from 240 unique primer combinations would represent all mRNA that were present in the original RNA sample (Liang and Pardee, 1992). PCR product visualization is conducted using polyacrylamide gel electrophoresis followed by the appropriate imaging. The images are then analyzed by comparing the relative intensities of bands produced from different experimental samples. Bands that show the difference in relative intensities among experimental samples represent the potential differentially expressed mRNA transcripts. The final phase of differential display is to identify the sequence of the transcript represented by the differentially displayed PCR product and to confirm that the transcript is truly differentially expressed. The steps are done by locating and excising the acrylamide gel containing PCR products of interest. The excised PCR products are purified from the gel and reamplified. Various techniques have been used for confirmation of the differential expression, including the use of reamplified PCR products as a probe for Northern hybridizations, spotting multiple reamplified PCR products on membranes for reverse dot-or slot-blot analysis, and cloning and sequencing reamplified PCR products so that gene specific primers may be designed for use in semi-quantitative PCR of cDNA. Two important advantages of the method are the ability to compare multiple experimental samples simultaneously

and to identify genes that are up-regulated or down-regulated from the experiment (Moody, 2001).

**2.6 Giant Tiger Shrimp**

Giant tiger shrimp (*Penaeus monodon*) is taxonomically classified as the member in Phylum Arthopoda; Subphylum Crustacea; Class Malacostraca; Subclass Eumalacostraca; Order Decapoda; Suborder Natantia; Infraorder Penaeidea; Superfamily Penaeoidea; Family Penaeoidea; Genus *Penaeus*; Species *Penaeus monodon* (Fabricius, 1798). Giant tiger shrimp can be distinguished from the other penaeids shrimp from the rostral characteristic of which the formula is 7-8+5-6.

Giant tiger shrimp is one of the most important economic species in Thailnad. The country earns thousands million baths annually from shrimp export (Office of agricultural economics and Customs department, 2005). Basically, shrimp farms are located mainly along the coastal areas where large amount of seawater is available. Since the market demand of shrimp has increased dramatically during the last few years, shrimp industry has been expanded uncontrollably. Consequently, the shrimp farms along the shoreline have become crowded and unavoidably confronted with the incurable diseases causing massive losses of shrimp culture production. As the shrimp aquaculture and farm management have been developed, shrimp culture can be operated successfully using water with very low salinity or even freshwater. Since there is no limit on water supply, shrimp culture area has been expanded and introduced deeper into the mainland where agricultural activities are heavily operated. The agricultural areas where shrimp farms are increasingly conducted are central, eastern, and southern Thailand (Limsuwan and Chanrachchakul, 2004). Researches on the shrimp aquaculture were relatively advanced, but information on the toxicity of xenobiotics is scarce. Table 2.3 shows export value of the shrimp which tended to decrease during 2002 to 2005. The information implied the massive losses of shrimp production with various suspected causes e.g. diseases, toxicity of contaminants, intolerance of shrimp to environmental stressors. In this study, therefore, the adverse effect of pesticides, which is one of the major chemicals used in agricultural activity but less concerned to aquaculture industry, to the shrimp has come to attention.

**Table 2.3** Giant tiger shrimp export from Thailand during 2002-2005.

| Month | 2002 | | 2003 | | 2004 | | 2005 | |
|---|---|---|---|---|---|---|---|---|
| | Quantity (MT) | Value (MB) | Quantity (MT) | Value (MB) | Quantity (MT) | Value (MB) | Quantity (MT) | Value (MB) |
| **Jan** | 11,345 | 3,894.80 | 13,360 | 4,671.66 | 11,746 | 3,145.25 | 5,625 | 1,510.76 |
| **Feb** | 10,821 | 3,763.43 | 11,453 | 3,916.07 | 12,606 | 3,356.08 | 4,193 | 1,098.32 |
| **Mar** | 12,578 | 4,260.60 | 11,594 | 3,890.99 | 4,610 | 1,332.69 | 4,537 | 1,248.03 |
| **Apr** | 12,308 | 4,026.19 | 11,230 | 3,895.05 | 5,782 | 1,785.85 | 3,603 | 968.76 |
| **May** | 14,655 | 4,946.44 | 12,594 | 4,120.20 | 7,082 | 1,937.04 | 4,313 | 1,194.83 |
| **Jun** | 15,545 | 5,468.02 | 12,446 | 4,089.82 | 8,414 | 2,780.45 | 6,623 | 1,749.64 |
| **Jul** | 14,285 | 5,019.06 | 14,055 | 4,563.97 | 9,902 | 3,142.45 | 6,857 | 1,885.88 |
| **Aug** | 17,295 | 5,741.69 | 15,731 | 5,036.36 | 8,327 | 2,595.77 | 6,678 | 1,870.81 |
| **Sep** | 19,808 | 7,196.31 | 17,988 | 5,611.11 | 10,498 | 3,358.83 | 7,339 | 1,946.05 |
| **Oct** | 21,265 | 8,041.29 | 18,057 | 5,358.97 | 10,853 | 3,321.91 | 6,864 | 1,857.45 |
| **Nov** | 18,939 | 6,982.56 | 11,857 | 3,454.28 | 9,797 | 2,997.04 | 6,618 | 1,817.32 |
| **Dec** | 12,206 | 4,485.87 | 10,832 | 2,974.41 | 8,904 | 2,458.51 | 6,162 | 1,605.64 |
| **Total** | **181,050** | **63,826.26** | **161,197** | **51,582.89** | **108,521** | **32,211.87** | **69,412** | **18,753.49** |

Source: Office of Agricultural Economics, Ministry of Agriculture and Cooperative