

## CHAPTER II

### LITERATURE REVIEWS

#### Organochlorine pesticides

##### Pesticides

Pesticides are substrates or mixture of substances intended for protection plants against molds, fungi and insect; therefore decreasing the percent of potential illness. Previously, pesticides are extracted from plants which naturally produced these chemicals towards repelling-off their predators. Later on, synthetic chemicals have been widely used as pesticide due to their ease of large scale production with lower cost (Perry *et al.*, 1998). Pesticides are toxic to living organisms in many ways and one of the concerning substances is organochlorine pesticides.

##### Organochlorine pesticide group

Organochlorine pesticides (OCPs) are insecticides composed primarily of carbon, hydrogen, and chlorine. They are stable organic compounds of very low water solubility and high lipophilicity. Some of them are highly persistent in their original forms or as stable metabolites in the environment long after application and in organisms long after exposure. Organochlorine pesticides are considered acting as environmental hormones, which disrupt reproductive cycle of humans and wildlife (NRC, 1999). Diverse group of organochlorine pesticides belonging to three distinct chemical classes include the dichlorodiphenylethane-, the chlorinated cyclodiene-, and also the chlorinated benzene- and cyclohexane-related structure (Table 2.1). From the mid-1940s through the mid-1960s, these compounds were used extensively in all aspects of agriculture and forestry, in

building and structural protection, and in human situations to control a wide variety of insect pests. The low volatility, chemical stability, lipid solubility, slow rate of biotransformation, and degradation properties that made these chemicals such effective insecticides also brought about their demise because of their persistence in the environment, bioconcentration, and biomagnifications within various food chains. The acquisition of biologically active body burdens in many wildlife species that, if not lethal, undoubtedly interfered with the reproductive success of the species (Klaassen, 1996).

Organochlorine pesticides were divided by structure into 3 groups namely dichlorodiphenylethanes, chlorinated cyclodienes, and hexachlorocyclohexanes (Klaassen, 1996).

#### Dichlorodiphenylethanes

##### *p,p'*-Dichlorodiphenyltrichloroethane (DDT)

DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane) is a pesticide that was once widely used to control insects on agricultural crops and insects that carry diseases like malaria and typhus, but is now used in only a few countries to control malaria. DDT still enters the environment because of its current use in other areas of the world. DDE is only found in the environment as a result of contamination by and degradation of DDT. DDD also enters the environment during the breakdown of DDT. Large amounts of DDT were released into the air and on soil or water when it was sprayed on crops and forests to control insects. DDT is semi-volatile and can be expected to partition into the atmosphere to reactions caused by sunlight. DDT and breakdown products are also present almost everywhere in the environment because they are persistent compound. DDT in the bottom sediment can also be absorbed by

some water plants and by the aquatic animals which consume those plants. DDT metabolites can be transported through food webs to top consumers such as humans (Thirakhupt *et al.*, 2006).

The cycle of evaporation and deposition may be repeated many times. As a result DDT, DDE, and DDD can be carried long distances in the atmosphere. Some DDT can enter the soil from waste sites. DDT, DDE, and DDD may occur in the atmosphere as a vapor or attach to solid particles in the air. Vapor phases of DDT, DDE, and DDD may be broken down in the atmosphere due to reactions caused by sunlight. The half-life of these chemicals in the atmosphere as vapors has been calculated to be approximately 1.5-3 days. DDT is broken down slowly to DDE and DDD by the microbial degradation. They stick strongly to soil, and therefore, generally, remain in the surface layers of soil. Some soil particles with attached DDT, DDE, or DDD may get into rivers and lakes through runoff. Only a very small amount, if any, will seep into the ground and migrate into groundwater. DDT disappears faster when the soil is flooded or wet than when it is dry. In tropical areas, DDT, DDE, and DDD may disappear in much less than a year. However, in some cases, half of it remains for 20, 30, or more years. In surface water, DDT binds to particles in the water, then settles and is deposited in the sediment. DDT is taken up by small organisms and fish in the water. It accumulates to animal in higher trophic level (ASTDR, 2002).

### Methoxychlor

Methoxychlor is used on agricultural crops and livestock, including animal feed, barns and grain storage bins. It does not readily evaporate into air or dissolve in water but can accumulate in some living organisms including algae, bacteria, snails, clams, and some fish. However, most animals convert

methoxychlor into other substances that are rapidly released from their bodies. Methoxychlor is efficient against flies, mosquitoes, cockroaches, and a wide multiplicity of other insects. It is used on agricultural crops and livestock, including animal feed, barns and grain storage bins. It does not readily evaporate into air or dissolve in water but once methoxychlor is in water, it usually binds to sediment or organic matter and settles to the bottom. In the soil, some methoxychlor is broken down by bacteria and other microorganisms, and some is by reactions to water or materials in soil. In air and water, some methoxychlor is usually broken down by sunlight. Methoxychlor can accumulate in some living organisms including algae, bacteria, snails, clams, and fishes (ASTDR, 2002).

#### Hexachlorocyclohexanes (HCH)

Hexachlorocyclohexane (HCH), formally known as benzene hexachloride (BHC), is a synthetic chemical that exists in eight chemical forms called isomers. The different isomers are named according to the position of the hydrogen atoms in the structure of the chemical (ATSDR, 2005). HCH has similar properties to other organochlorine insecticides and HCH are classified into alpha ( $\alpha$ ), beta ( $\beta$ ), gamma ( $\gamma$ ), and delta ( $\delta$ ) isomers. They were produced and used as an insecticide on fruit, vegetables, and forest crops, and also animals and animal premises. They have been found in the soil and surface water at hazardous waste sites because of their persistence in the environment. In the air, the different forms of HCH can be present as a vapor or attached to small particles such as soil and dust. The particles may be removed from the air by rain or degraded by other compounds found in the atmosphere. HCH can remain in the air for long periods and travel great distances depending on the environmental condition. In soil, sediments, and water, HCH is broken down to

less toxic substances by algae, fungi, and bacteria, but this process can take a long time (ATSDR, 2005).

### Chlorinated cyclodienes

Cyclodiene insecticides are cyclic hydrocarbons having a chlorine substituted methanobridge structure. Aldrin, dieldrin, heptachlor, chlordane, and endosulfan are examples of this group. Major uses are aldrin for the control of a wide range of soil pests, grasshoppers, and certain cotton insects. Another example is endrin which controls a wide range of foliage pests of cotton, rice, tobacco, maize sugarcane, and fruit trees. Endrin and endosulfan are very toxic in vertebrate, but limited biological persistence. Aldrin is readily metabolized to dieldrin by both plants and animals, so aldrin residues are rarely found in foods and animals and reside in small amounts. They were also used in dips and sprays to control ectoparasites of livestock and were widely used as seed dressings for cereals and other crops. The use of aldrin, dieldrin, and heptachlor for the latter purpose has caused very serious ecological consequences through food chains and food webs including contamination in soil, water, and groundwater (Thirakhupt *et al.*, 2006).

### Aldrin and Dieldrin

Aldrin and dieldrin are the common names of two structurally similar compounds that were once used as insecticides. They are chemicals made in the laboratory and do not occur naturally in the environment. Aldrin and dieldrin slowly evaporate in the air. Aldrin evaporates more readily than dieldrin. Both aldrin and dieldrin have mild chemical odors. Residues have been found in soil, water, and buildings where these compounds were used to kill termites. They also have been found in plants and animals near the hazardous

waste sites Sunlight, other physical factor, and microorganisms in the environment can convert aldrin to be dieldrin. Therefore, dieldrin can be found in places where aldrin was originally released. Dieldrin in soil or water is degraded very slowly. It sticks to the soil and may exist there unchanged for many year. Water does not easily wash dieldrin off siol. Dieldrin does not dissolve in water very well and is, therefore, not found in water at high concentrations. Most dieldrin in the environment has attached to soil and to sediments at the bottom of lakes, ponds, and streams. Many carnivorous animals have level of dieldrin in their fat tissues much higher than herbivorous animals (ASTDR, 2002).

### **Endosulfan**

Endosulfan is a man-made insecticide. It is used for control of a number of insects on food crops such as grains, tea, fruits, and vegetable; and also on nonfood crops such as tobacco and cotton. Endosulfan enters the air, water, and soil during the manufacturing process or when used as a pesticide. Endosulfan generally degrades down within a few weeks and released into the soil. It is usually attached to soil particles and found in the soil at hazardous waste sites. Most endosulfan in surface water is attached to soil particles floating in the water or attached to soil at the bottom. Animals inhabiting endosulfan-contaminated area can build up endosulfan in their bodies (ASTDR, 2000).

### **Endrin**

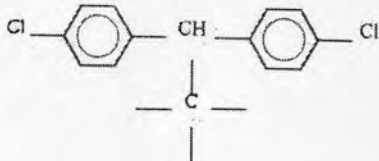
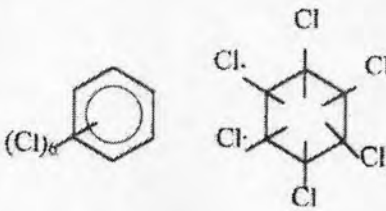
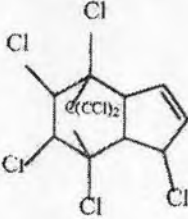
Endrin was used as a pesticide to control insects, rodents, and birds. Little is known about properties of endrin aldehyde, and impurity and the breakdown product of endrin, or endrin ketone, which is a product of endrin when it is exposed to sunlight. It's more likely to cling to the bottom sediment of

rivers, lakes, and other body of water. The persistence of endrin in the environment depends highly on local conditions. However, the amount of endrin is broken down to endrin aldehyde is very small (less than 5%) and it is not know what happens to endrin aldehyde once they are released to the environment (ASTDR, 1996)

### Heptachlor

Heptachlor is a synthetic chemical that was used in the past for killing insects in homes and on food crops. Heptachlor epoxide is a breakdown product of heptachlor that was converted by microorganisms in the environment this profile describes these chemicals together because about 20% of heptachlor is changed within hours into heptachlor epoxide in environment and in the human body. They can be found in the soil and the air around buildings treated for termites. It has dissolved in surface water or groundwater, or evaporated into the air also found in plants and animals near hazardous waste sites. Heptachlor epoxide is a broken down very slowly in the environment. It can exist in the soil and water for many years. Both heptachlor and heptachlor epoxide are accumulated in animals and humans. Some studies show that heptachlor epoxide can exist in fat tissues for 3 years after exposed (ASTDR, 2007).

Table 2.1 Structural classification of organochlorine pesticides

Organochlorine pesticide group	Chemical structure	Examples
Dichlorodiphenylethanes		DDT, DDD, DDE, Dicolfol, Perthane, Methoxychlor, Methlochlor
Chlorinated Benzenes Cyclohexane		BHC, HCH, Lindane ( $\gamma$ -BHC)
Cyclodienes		Aldrin, Dieldrin, Heptachlor, Chlordane, Endosulfan

Source: Klaassen, 1996

### Organochlorine pesticide contamination

Pesticides are toxic to living organisms in many ways and one of the concerning substances is organochlorine pesticides. The reason behind this is that they are water insoluble and present in the environment for a long time. Moreover, organochlorine pesticides, being subjected to slow chemical and biological degradations, can be accumulated in organisms and biomagnified along the food chain (Ahlborg *et al.*, 1992). Although some organochlorine pesticides such as DDT, dieldrin, and lindane, have been banned in Thailand for more than ten years, most recent studies have reported that these pesticide residues can



still be detected in crops, water, sediment and biota of many aquatic ecosystems (Pipithsangchan *et al.*, 1997; Thapinta and Hudak, 2000). However, due to the ongoing use of wide range of organochlorine pesticides, human exposure to OCPs in the developing countries in Asia was between 5 and 100 times higher than in developed countries (Kannan *et al.*, 1997).

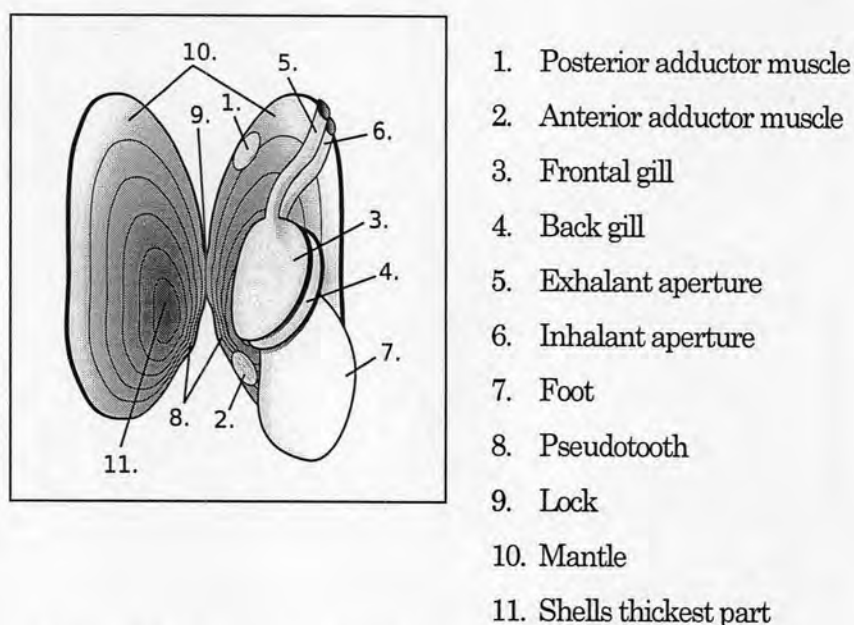
### Freshwater Mussels

Mussels are in a group of invertebrate animals called mollusks. The mussels are classified in Class Bivalvia of Phylum Mollusca. There are about 8,000 described species of aquatic mollusks, almost in Order Unionoida, a sole freshwater group. These soft-bodied animals are enclosed by two hard shells made mostly of calcium and connected by a hinge. The bivalves possess a hinge which is most developed posterior of the beaks or umbones. The shells are typically covered by a thick skin or periostracum which varied in colors and, sometimes, rays on the disk depending on the species. They are also referred to as clams, naiads, and unionids. All freshwater mussels live in the sediments of rivers, streams, and lakes. A part of their life cycle were pass through a parasitic larval stage, living on the fins, gills, and bodies of fishes (Brandt, 1974).

In a typical bivalve, the body is laterally compressed, entirely enclosed within a shell of two valves hinged along the mid-dorsal line. The mantle lobes and mantle cavity are correspondingly large. The foot is blade-like and directed anteriorly. There is no head and, aside from the osphradium, the mantle margin is the principal site of receptors monitoring external signals. Almost all bivalves are suspension feeders, and the radula has disappeared.

Anterior and posterior adductor muscles extending transversely between the valves bring about valves closure. They operate antagonistically to the

nonliving, dorsal hinge ligament, which causes the valves to open. Most bivalves are filter feeders, utilizing the gill as the filter. In the evolution of this mode of feeding, the gill filaments become lengthened and folded, greatly increasing the surface area. They have separate sexes, but some are hermaphroditic. In most bivalves the gonoducts open directly to the exterior. Fertilization is external or may occur in gill brood chambers (Unionoda and Pisidiidae; Brandt, 1974).



**Figure 2.1** Organs of freshwater mussels,  
([http://en.wikipedia.org/wiki/Freshwater\\_Pearl\\_Mussel/](http://en.wikipedia.org/wiki/Freshwater_Pearl_Mussel/) 29 June 2006)

Mussels spend its life anchored in rivers or lake sediments. The vast majority of them are found in streams. Their movement is through either muscular feet or powerful flood currents. A mussel captures oxygen and microscopic food particles in flowing water through filtration, continuously pump water through their bodies. Water enters through the incurrent or branchial siphon and exits via the excurrent or anal siphon. This pumping process allows the mussel to filter food from the water. The food consists of detritus, organic matter found on the stream, and planktons (Dillon, 2000). Mussels are long lived species with some living more than 10 years (Brandt, 1974). Others have been

recorded as over 100 years old. There are however pollutants that may reduce this life expectancy.

Many species of freshwater mussels have been used as a sentinel species to predict the health of the environment. Mussels respond to changes in water quality. Gradual or sudden mussel die-offs are reliable indicators of water pollution problems and other environmental health concerns. Stable, diverse mussel populations generally indicate clean water and a healthy aquatic environment (Jernelov, 1996). They serve as an important aspect of the aquatic ecosystem which they occupy. However, the number of mussels has been declining such that many are now considered as endangered species which has sparked renewed interest in their study.

Factors contributing to the decline of mussels are overharvest, siltation of their habitat, agriculture, poor land management, pollution by herbicides, pesticides, and other chemicals. Freshwater mussels have a high ecological value. They are an important food source for many other animals. More importantly, they serve as bioindicators since they are sensitive to pollutants such as heavy metals, pesticides, agricultural nutrients, heavy loads of fine silts (Jernelov, 1996). It is inevitable that mollusks are going to be exposed to water born chemicals. Since mollusks are not very good at biodegrading these pollutants, exposure to these chemicals pose a threat to their lives.

The mussels are widely used as sentinel organism because it can attaches itself to the toxic substrate, exposes with toxicant by filter feeding, and live in the benthic of their habitats, such as coastal, river, lake and canal. Bayen *et al.*, (2004) used green mussel, *Perna viridis*, the marine bivalves commonly found in Asia Pacific water to measure the bioaccumulated levels of OCPs in marine environment in Singapore. In England, blue mussel, *Mytilus edulis*, have been used

for monitoring the organochlorine contamination in the Mersey estuary for a numbers of years (Conner *et al.* 2001). Freshwater mussels has also been used for assessment of the Persistent Organic Pollutants (POPs) contamination, heavy metal and organotin-compounds in freshwater environment including used to evaluate DDT bioaccumulation in Lake Maggiore at Italy (Binelli *et al.*, 2004).

Freshwater mussel collected from Khlong 7, Rangsit agricultural area

*Pilbryconcha exilis exilis*



Figure 2.2 *Pilbryconcha exilis exilis* collected from the study site at Khlong 7, Rangsit agricultural area, Pathum Thani Province.

Description: shell elongately liguiform but less narrow than the other species; with strong concentric growth lines, smooth and shining, olive green, with rounded anterior end pointed podium, ventral margin only slightly arched, in very large specimens almost straight in the middle. Umbones not prominent, eroded; very young specimens show an umbonal sculpture which consists of 3-5 short and straight furrows only. Hinge small, without any dentition or with a very feeble, rudimentary pseudocardinal in each vale. Pallial line shallow; anterior muscle scar moderately deep, posterior very shallow. Nacre within bluish white (Brandt, 1974).

Size: Length 80-120 mm; Aperture 44-58 mm; Diameter 17-26 mm

*Hyriopsis (Limnoscapha) desowetzi*



Figure 2.3 *Hyriopsis (Limnoscapha) desowetzi* collected from the study site at Khlong 7, Rangsit agricultural area, Pathum Thani Province.

Description: shell ovate, inflated, with high posterior and small anterior wing which points upwards. Texture of the shell moderately thick but thinner than that of the type species. The thick periderm is greenish when young, but turn brownish or even blackish with age. The dorsal line is straight and slants from the tip of the wing to the tip of gonium. The posterior margin slopes to the angled podium where it turns into the ventral margin which is regularly arched to the gonium. This is produced into a short, beaklike anterior wing. The umbones are placed in the anterior third of the shell. They are not prominent and always eroded. Young specimens show some concentric furrows on the umbones; there are 1 or 2 more or less sharp areal folds running from the umbones to the podium. The lamelliform lateral teeth are comparatively short and somewhat curved. The pseudocardinals in the left valve are placed behind each other; the first is thicker but less sharply ridged than the second. The nacre is whitish. The pallial line is not interrupted. The anterior muscle scars are deep and not confluent, the posterior are shallow (Brandt, 1974).

Size: Length 90-115 mm; Aperture 68-80 mm; Diameter 33-40 mm

*Uniandra contradens ascia*

Figure 2.4 *Uniandra contradens ascia* collected from the study site at Khlong 7, Rangsit agricultural area, Pathum Thani Province.

Description: oval-subtrapezoidal, inequilateral, moderately thick, tumid, glossy, brownish-olive, with concentric wrinkles which may sometimes be restricted to the umbonal area only. Ventral and dorsal margin convex, the former rising posterior, the latter slightly sloping; anterior end rounded, posterior end biangulate or truncate, rarely produced into a beak. There are two weak ridges running from the umbones to the posterior part of the shell. Ligament straight or somewhat curved, brownish, placed behind the umbones. Teeth comparatively strong, typical for the genus (Brandt, 1974).

Size: Length 45-75 mm; Aperture 30-45 mm; Diameter 24-35 mm

### Biomarker

Biomarkers are fundamental challenge in environmental toxicology relating the presence of a chemical in the environment with a valid prediction of ensuing hazard to potential biological receptors. Adverse health effects in biological receptors begin with exposure to a contaminant and can progress to damage or alteration in function of an organelle, cell, or tissue. Environmental risk was assessed by chemical residue determination in samples of environmental media, combined with comparison to toxicity observed in species

in contact with the media. Chemical residue analysis of tissues containing the biological receptor may also be performed (Mitchelmore *et al.*, 2006).

The National Academy of Sciences defines a biomarker or biological marker as a xenobiotically induced alteration in cellular or biochemical components or processes, structures, or functions that is measurable in biological system or sample (NRC, 1987). Biomarkers can be broadly categorized as markers of exposure, effects, or susceptibility (Klaassen, 1996)

### **Biomarkers of Exposure**

The presence of a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured within a compartment of an organism can be classified as a biomarker of exposure (ATSDR, 1994). Biomarkers of exposure are used to predict the dose received by an individual, which can then be related to changes resulting in a disease state, an example of this is the G-to-T transversion created following the formation of a N7 guanine adduct by benzo[a]pyrene 7,8-dihydrodiol-9,10-epoxide (BPCE) (Shibutani *et al.*, 1993).

### **Biomarkers of Effect**

Biomarkers of effect are defined as measurable biochemical, physiologic, behavioral, or other alterations within an organism that depending on their magnitude can be recognized as an established or potential health impairment or disease (ATSDR, 1994). A biomarker of effect result must be able to stand alone and does not need chemical analysis or additional biological tests for confirmation.

Examples of such biomarkers include inhibition of brain cholinesterase by organophosphate or carbamate insecticides, induction of delta aminolevulinic

acid synthetase and inhibition of aminolevulinic acid dehydratase by lead and eggshell thinning by DDE. Less specific biomarkers are also well validated, but they have wider applications and tend to respond to broader classes of chemicals such as the induction of mixed function oxidases. For example, the induction of cytochrome P4501 (CYP1A1) enzymes in fish liver is generally recognized as a useful biomarker of the exposure of fish to anthropogenic contaminants, but not compound specific.

### Biomarkers of Susceptibility

Biomarkers of susceptibility are endpoints that are indicative of an altered physiologic or biochemical state that may predispose the individual to impacts of chemical, physical, or infectious agents. For example, low-level exposure to a cytochrome P4501A1 or 1A2 inducer may result in elevated enzyme activity in wildlife but no observable adverse effects. Such elevations in enzyme activity in humans have been linked to greater risk of a number of cancers or decreases in conjugation enzymes and their high-energy substrates. A number of xenobiotic compounds inhibit the activities of the immune system and thus increase susceptibility to infectious agents, parasites and cancer.

Among the several of biomarkers used to study the effects of animal exposure to environmental contamination. The biomarker is used to include almost any measurement reflecting an interaction between biological system and environmental agent which may be chemical, physical and biological. The biomarker may also make it possible to identify susceptible groups or individuals a risk of exposure. The biomarkers of freshwater mussels responded the organochlorine pesticides such as aldrin, DDT, and its metabolites, were lysosomal membrane damage and histopathologic in gonad (Werner *et al.*, 2004).



The levels of estrogen present in municipal were found to induce the expression of vitellogenin in fish and bivalves (Gagné *et al.*, 2001). The specific biomarker provides important biological information on the potential impact of xenobiotics in the health of organisms and ecosystem. Various biomarkers have been measured in mollusks, specifically in *Elliptio complanata*, such as vitellogenin, metallothionein, lipid peroxidation, protein-free DNA and glutathione S-transferase (Won *et al.*, 2005)

### Vitellogenin

Vitellogenin (vtg) is a protein that appears in the blood of sexually maturing female oviparous vertebrates. It is produced by the liver in response to circulating estrogen, released into the bloodstream, taken up by growing oocytes, and chemically modified to form a suite of egg yolk protein, a large phospholipo glycoprotein is the precursor of major yolk proteins in most oviparous animals. The term "vitellogenin" was first used by Pan *et al.* (1969) to define the female specific blood protein of insects that had common functional characteristics and were closely related in composition to the yolk protein.

The term vitellogenin is also applied to the corresponding proteins in oviparous and viviparous non-mammalian vertebrates. A common characteristic of vitellogenin in most egg-laying animals is that they are synthesized in a tissue, stage-and sex-specific manner, outside of the final site of deposition. The major sites of synthesis of vitellogenin are the intestine, the fat body, and the liver, respectively for nematodes or echinoderms, insects and non-mammalian vertebrates. Vitellogenins are produced in large amounts by female tissues, accumulated by developing oocytes through receptor-mediate endocytosis, and eventually used as a source of nutrients for developing embryos (Sappington and

Raikhel, 1998). In invertebrates, such as insects and crustaceans, the vitellogenins are termed vitellin (vn) once they are deposited in the oocyte.

Vitellogenin is normally produced by females in response to normal cycles of estradiol during oogenesis. The gene for vitellogenin is also present in the livers of males but it is normally silent. Upon exposure to estrogen or to an estrogen mimic, the gene is turned on and vitellogenin is synthesized. After synthesis, it is exported into the blood where, in males, it remains until it is degraded or cleared out by the kidneys. In females, vitellogenin is taken up by the developing oocyte through receptor mediated endocytosis. As more Vtg protein and gene sequences become available due to development of molecular techniques, the term yolk protein precursor (YPP) has come into use to replace the classical meaning of vtg. This is because in various species, the yolk protein precursors are not vitellogenin but is one of several other classes of protein. The major yolk proteins are homologous to lipoprotein lipases, but not other vitellogenin proteins.

#### Vitellogenin as a bioindicator

Vitellogenin, the egg yolk precursor protein, has become a popular biomarker for measuring exposure of oviparous animals to estrogen or estrogen mimics. The effects of pollutants in aquatic environments on endocrine and reproductive systems are due to their mimicking or antagonizing the effects of hormones, altering the patterns of synthesis and metabolism of hormones and modifying hormone receptor levels. Many pollutants mimic the action of endogenous estrogens and include synthetic chemicals such as pesticides, polychlorinated biphenyls (PCBs), and household products (surfactants). These xenoestrogens can bind the estrogen receptor and may induce a hormone-

dependent response such as vitellogenesis (Sumpter and Jobling, 1995). In males, endogenous estrogen levels are so low that in general vitellogenesis is not supported, but maybe induced by exposure to exogenous estrogen. In most female vertebrates, the natural response to estrogen is restricted to the time of the year when oocytes are developing. Therefore, exposure of most males at anytime, or of females outside the breeding season to adequate amounts of exogenous natural or xenoestrogens, will induce the vitellogenin gene and vitellogenin production via a classical receptor-based mechanism. This can provide an index of xenoestrogen exposure *in vivo* (Jobling *et al.*, 1995). Vitellogenin have been widely used as a biomarker of exposure to estrogenic compounds in aquatic environments, mostly in fishes (Marin and Matozzo, 2004). Furthermore, an impaired or abnormal vitellogenic response *in vivo* or *in vitro* can be used to assess other natural hormones, xenobiotics, or endocrine disruptors using the same or parallel and interacting steroid receptor systems. So far most endocrine disruption studies have used fish, as representing the largest and most diverse group of aquatic vertebrates. They are thus excellent models for assessing the effects of aqueous contaminants on biological function including vitellogenesis.

Although invertebrates can synthesize vtg, it is not known whether vertebrate estrogens or other steroids, with the exception of ecdysone, are involved in its regulation. However, vertebrate types of steroids have been chemically identified in insects and many are thought to have originated from a dietary source since insects cannot synthesize cholesterol. Steroids identified include estrone, estradiol, estriol, testosterone, dihydroxytestosterone, androsterone, pregnenolone, progesterone and cortisol. However, there is no

evidence that these compounds play a role in sexual differentiation or reproduction of invertebrates.

### Glutathione S-Transferase

Glutathione S-transferase (GST) is a family of enzymes that are important in the detoxification of many different xenobiotics in animals and plant. GST represents a major group of detoxification enzymes, utilize glutathione in reactions contributing to the transformation of a wide range of compounds, including carcinogens, therapeutic drugs, and products of oxidative stress. These enzymes play a key role in the detoxification of such substances. The enzymes protect cells against toxicants by catalyzing the reaction of glutathione with an acceptor molecule to form an S-substituted glutathione, S as sulfur (Habig *et al.*, 1974).

The three principal routes of xenobiotic detoxification in animals and plants are enzymatic hydrolysis, oxidation, and conjugation of intrusive compounds into less toxic, more water-soluble metabolites (Hughes, 1996). In insects the enzymes responsible for the reactions become mechanisms for insecticide resistance when they are present in higher than normal amounts or have higher affinity for the poison. One of detoxification enzyme is the glutathione S-transferases (EC 2.5.1.18, GST) to play a physiological role in initiating the detoxication of potential alkylating agents including organochlorine pesticide, heavy metal, and pharmacologically active compounds. These enzymes catalyze the reaction of such compounds with the -SH group of glutathione, thereby neutralizing their electrophilic sites and rendering the products more water-soluble. There is a voluminous amount of literature which deals with

individual glutathione transferase reactions in relatively crude enzyme preparations from rat liver.

Glutathione S-Transferase are a group of enzymes of all eukaryotic species possess multiple cytosolic and membrane-bound GST isoenzymes, each of which displays distinct catalytic as well as noncatalytic binding properties: the cytosolic enzymes are encoded by at least five distantly related gene families (designated class alpha, mu, pi, sigma, and theta GST), whereas the membrane-bound enzymes, microsomal GST and leukotriene C4 synthetase, are encoded by single genes and both have arisen separately from the soluble GST. The biological control of these families is complex as they exhibit sex-, age-, tissue-, species-, and tumor-specific patterns of expression. In addition, GST are regulated by a structurally diverse range of xenobiotics and, to date, at least 100 chemicals have been identified as inducer of GST. A significant number of these chemical inducers occur naturally and, as they are found as non-nutrient components in vegetables and citrus fruits, it is apparent that humans are likely to be exposed regularly to such compounds. Many inducers, but not all, affects transcriptional activation of GST genes through both the antioxidant-responsive element, the xenobiotic-responsive element, and the glucocorticoid-responsive element. Many of the compounds that induce GST are themselves substrates for these enzymes, or are metabolized, by cytochrome P-450 monooxygenases, to compounds that can serve as GST substrates, suggesting that GST induction represents part of an adaptive response mechanism to chemical stress caused by electrophiles. It also appears probable that GST are regulated *in vivo* by reactive oxygen species, because not only are some of the most potent inducers capable of generating free radicals by redox-cycling. The induction of GST would appear to represent an adaptive response as these enzymes detoxify some of the toxic

carbonyl-, peroxide-, and epoxide-containing metabolites produced within the cell by oxidative stress. The mechanisms responsible for over expression of GST include transcriptional activation, stabilization of either mRNA or protein, and gene amplification. Hoarau (2004) reported the GST activity in clam, *Ruditapes decussatus*, exposed to DDE and methoxychlor.

### Study area

Rangsit agricultural area is located at the central part of Thailand in Pathum Thani Province. It has an irrigation-network-system, consisting of 14 sub-canals (Khlung) which are divided into two parts, the upper part and the lower part. The study area is situated at Khlung 7 canal, a 20 km-man-made sub-canal, on the upper part of the irrigation-network-system. It links Raphi Phat canal at the upstream side and Rangsit-Prayulasakdi canal at the downstream side. The Rangsit irrigation-network-system has supported various agricultural activities such as paddy fields, the main sort of agricultural practice approximately 70 % of the total of the province's land use (Office of Agricultural Economics, 2002), vegetable farms, fruit orchards, and fisheries.