จุลพยาธิวิทยาของตับปลาข้าวเม่า *Parambassis siamensis* บริเวณพื้นที่เกษตรกรรมคลอง 7 จังหวัดปทุมธานี

นางสาว จิตนิภา สำอางศรี

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HISTOPATHOLOGY OF SIAMESE GLASSFISH *Parambassis siamensis* LIVER AT KLONG 7 AGRICULTURAL AREA, PATHUM THANI PROVINCE

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Zoology

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จิตนิภา สำอางศรี : จุลพยาธิวิทยาของตับปลาข้าวเม่า *Parambassis siamensis* บริเวณพื้นที่เกษตรกรรมคลอง 7 จังหวัดปทุมธานี. (HISTOPATHOLOGY OF SIAMESE GLASSFISH *Parambassis siamensis* LIVER AT KLONG 7 AGRICULTURAL AREA, PATHUM THANI PROVINCE) **อ. ที่ปรึกษา : รศ.ดร. กิ่งแก้ว วัฒนเสริมกิจ , อ.ที่ปรึกษาร่วม : ผศ. ดร. กำธร ธีรคุปต์ จำนวนหน้า 144 หน้า.** ISBN 974-53-1483-8.

ศึกษาการเปลี่ยนแปลงเนื้อเยื่อตับของปลาข้าวเม่า *Parambassis siamensis* และปริมาณตกค้างของสารปราบศัตรูพืชที่ใช้ในการป้องกันและกำจัดศัตรูพืช กลุ่มออร์กาโนคลอรีนในน้ำที่บริเวณพื้นที่เกษตรกรรมคลอง 7 จังหวัดปทุมธานี เก็บตัวอย่างน้ำตั้งแต่เดือน มีนาคม ถึง เดือน ธันวาคม 2547 และเก็บตัวอย่างปลาข้าวเม่าในเดือน มีนาคม และ เมษายน 2547 (ฤดูร้อน) และในเดือน พฤษภาคม ถึง สิงหาคม 2547 (ฤดูฝน) การศึกษาการเปลี่ยนแปลงของเนื้อเยื่อจากสไลด์ถาวร และการศึกษาปริมาณไขมันด้วยการย้อม Oil red O ปริมาณไกลโคเจนด้วยการย้อม PAS เก็บตัวอย่างน้ำจาก 3 บริเวณ มาทดสอบหาสารฆ่าแมลงกลุ่มออร์กาโนคลอรีน 17 ชนิด ด้วยแก๊สโครมาโตกราฟี จากการศึกษาพบสารปราบศัตรูพืช ได้แก่ α BHC, γ BHC, β BHC, heptachlor, δ BHC, aldrin, heptachlor epoxide, endosulfan I, 4, 4'-DDE, dieldrin, endrin, 4, 4'-DDD, endosulfan II, 4, 4'-DDT, endrin aldehyde, endosulfan sulfate และ methoxychlor ในจำนวนสารปราบศัตรูพืชที่ศึกษาครั้งนี้ พบเอ็นโดซัลเฟน และอนุพันธ์ของเอ็นโดซัลเฟนยังคงตกค้างในปริมาณสูง ผลการศึกษาชี้ให้เห็นว่ามีการใช้สารเหล่านี้ในบริเวณแหล่งเกษตรกรรมนี้ และพบว่ามีปริมาณสารพิษตกค้างไม่เกินค่ากำหนดที่อนุญาตให้มีได้ในน้ำบริโภค (MAC) นอกจากนี้ยังพบว่ามีการใช้ในปริมาณมากในช่วงฤดูฝน

การศึกษาพยาธิสภาพของเนื้อเยื่อตับปลา ในคลอง 7 ช่วงฤดูร้อน และช่วงฤดูฝน พบว่ามีการเปลี่ยนแปลงคล้ายคลึงกัน และมีความรุนแรง จากน้อยไปจนถึงความรุนแรงมาก ดังนี้ มีการสะสมแวคคิลโอลไขมันและไฮยาลินแกรนูลในเซลล์ตับ การเกิดเลือดคั่งในช่องไซนูซอยด์ การสลายของนิวเคลียส การหลุดของเซลล์เยื่อชั้นในของหลอดเลือด และการตายของเซลล์ตับในลักษณะที่เป็นกลุ่มและแพร่กระจายรอบหลอดเลือด ผลการศึกษาทางฮีสโตเคมีพบว่าตับปลากลุ่มคลอง 7 ในฤดูร้อนและในฤดูฝน มีการสะสมของแวคคิวโอลไขมัน และ ไกลโคเจนจำนวนมาก ส่วนเนื้อเยื่อตับปลาที่ได้รับสารเอ็นโดซัลเฟนที่ทุกระดับความเข้มข้น (0.01, 0.06, 0.25 และ 1 μg/L) มีการเปลี่ยนแปลงเนื้อเยื่อตับเช่นเดียวกับปลาในฤดูร้อนและฤดูฝน และมีความรุนแรงน้อยกว่า จำนวนแวคคิวโอลไขมันและ ไกลโคเจนในเซลล์ตับลดลงทุกระดับความเข้มข้น ทั้งนี้สรุปได้ว่าน้ำคลองในพื้นที่เกษตรกรรมคลอง 7 จังหวัดปทุมธานี มีสารพิษที่ก่อให้เกิดการเปลี่ยนแปลงพยาธิสภาพและฮีสโตเคมีของเนื้อเยื่อตับปลาข้าวเม่า

ภาควิชา.....ชีววิทยา..... ลายมือชื่อนิสิต.....

สาขาวิชา.....สัตววิทยา..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2547..... ลายมือชื่ออาจารย์ทีปรึกษาร่วม.....

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KEY WORD: *Parambassis siamensis* / HISTOPATHPLOGY / SIAMESE GLASSFISH /FISH / KLONG 7/ ORGANOCHLORINE PESTICIDE / GAS CHROMATOGRAPHY

CHITNIPAR SAM-ANGSRI : HISTOPATHOLOGY OF SIAMESE GLASSFISH *Parambassis siamensis* LIVER AT KLONG 7 AGRICULTURAL AREA, PATHUM THANI PROVINCE. THESIS ADVISOR : ASSOC. PROF. KINGKAEW WATTANASIRMKIT, Ph.D., THESIS CO-ADVISOR : ASST. PROF. KUMTHORN THIRAKHUPT, Ph.D., 144 pp. ISBN 974-53-1483 -8.

Histological alteration of the liver tissue of the Siamese glassfish *Parambassis siamensis* and organochlorine pesticide residues in water at Klong 7 agricultural area, Pathum Thani Province were studied. Water were sampled during March to December 2004 and fish were collected during March and April 2004 (summer) and May to August 2004 (rainy season). Histological alteration was studied on the H&E staining permanent slides preparation. Lipid droplet deposits were studied with Oil red O staining technique. Glycogen deposits were studied with PAS staining technique. Water samples were collected from 3 sites for determining organochloine residues with GC-μECD, using 17 types of universal standards. Organochloine peticide residues; α BHC, γ BHC, β BHC, heptachlor, δ BHC, aldrin, heptachlor epoxide, endosulfan I, 4, 4'-DDE, dieldrin, endrin, 4, 4'-DDD, endosulfan II, 4, 4'-DDT, endrin aldehyde, endosulfan sulfate, and methoxychlor were found. Among these residues, endosulfan and its derivatives were in highest concentration. The results indicate the previous used of organochlorine compounds in the area. However, the residues of each compound did not exceed the Maximum Allowable Concentration in drinking water standards. Moreover, most organochlorine pesticide residues in water were found higher in rainy season than in summer.

Histopathology of the Siamese glassfish liver at Klong 7 in summer and rainy season exhibited the same histological changes. The damages revealed varieties of changes from mild to severe i.e. fat droplet and hyaline granule accumulation, sinusoid dilatation and blood congestion, pyknotic nucleus and karyolysis, detachment of endothelial cell from blood vessels, focal and diffuse necrosis near blood vessels. The histochemical study of fish liver at Klong 7 in summer and rainy season showed the increase of both lipid droplets and glycogen deposits. Fish treated with endosulfan (0.01, 0.06, 0.5, and 1 μg/L) showed histological alteration of liver less than the fish collected from Klong 7 both in summer and rainy season. The histochemical study in endosulfan treated fish showed a reduction of both lipid droplets and glycogen deposits. It can be concluded that the water from Klong 7 agricultural area, Pathum Thani Province has the potential to cause histopathological and histochemical alteration in fish liver.

Department……Biology…….. Student's signature.....

Field of study…….Zoology……. Advisor's signature.....

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CHAPTER I

INTRODUCTION

Nowadays, there are many types of pesticides being produced and distributed around the world. About 314 of them have been imported into Thailand since the end of World War II. In B.E.2520 (C.E.1977) the total import of toxic or hazardous substances was 7,494 tons while in B.E.2544 (C.E.2001) the figure rose up to 37,039 tons (Misnistry of Agriculture and Cooperatives, 2002). Previous statistics showed that the trend of pesticide used has been increasing. In addition, pesticides have been imported for agricultural use and the focus is on increased productivity in the agricultural part. However, toxicity of pesticides can cause harmful effects on all living things when misused or when precautionary measures are lacking. It has impact, directly and indirectly, both on users and people involved in the application area, as well as the environment.

For the period of the past decade, Thailand enjoyed economic growth, particularly in the agricultural and industrial parts. The government has attempted to make Thailand a newly industrialized country (NIC). There is still the essential and the desperate need, however, to become domestically self sufficient in food production for export and to supply the national industries. Agricultural development, particularly the development of the agro-industry and export, requires modern technology. It has become unavoidable to significantly increase the use of pesticides. Therefore, the rise in export figures indicates that pesticides have been increasingly used both in terms of quantity and variety, especially highly hazardous substances, which have harmful and toxic impacts. The harmful effects have fallen on the users (farmers), workers in the manufacturing plants and others who come into contact with pesticides.

Since the early 1950s DDT has been extensively used in Thailand as malaria repellent and as an agricultural pesticide. During the last decade, the use has been successively limited and was finally banned for all applications in 1994 (Kumblad et al., 2001). In spite of this, persistance of the organochlorine pesticides are still circulating in

Thailand ecosystems that are simultaneously being exposed to the increase of pollution loads resulting from rapid population growth, urbanization and agroindustrial development. Moreover, organochlorine compounds such as DDT (dichlorodiphenyltrichloroethane), HCB (benzene hexachlorine), and polychlorinated biphenyls (PCBs) have been found in a wide range of environmental media and biota because of their lipophilic properties, persistence in the environment, and potential for magnification in the food chain (Aurigi et al., 2000).

Although DDT and other pesticides for agricultural use were banned for manufacture and use in many developed countries during 1970s (Das et al., 2002), they are still used in some lands and residue levels can still be recorded in various crops indicating present usage on agricultural lands. It is not surprising, therefore, that those pesticides find their way into ponds, streams, and rivers.

These pesticides are known to contaminate the water, sediment, and aquatic organisms in riversides. Aquatic animals can accumulate many chemicals in high concentrations even when the ambient environmental contamination levels are low, the processes call biomagnifications. The aquatic environment is always being contaminated with toxic chemicals from industrial, agricultural, and domestic activities. Such pesticides can be one of the major classes of toxic substances used in Thailand for management of pests in agricultural lands and control of insect vectors of human disease. The runoff from treated areas enters the rivers and aquaculture ponds that are supplied by river water, therefore they are contaminated by pesticides.

The Siamese glassfish *Parambassis siamensis* is a common fish in rivers of Thailand. Once a toxicant enters an organism, several biochemical processes will be changed, causing cell and tissue damages. Pesticide residues produce an adverse impact on the aquatic biota including fish. Continuous accumulation of pesticides may cause several changes in fish, altering histopathological condition of the liver (Klaassen, 2001). Consequently, the liver is the organ that suffers serious morphological alterations if exposed to pesticides.

Klong 7, located in Nong Sua District, Pathum Thani Province, is one of 14 sub-canals that have been used to irrigate Rangsit agricultural areas for more than 100 years. This area is undoubtedly one of the most contaminated spots of pesticides in the central plain of Thailand. Because of these multiple uses, pesticide contamination of Klong 7 fish may have adversely impact. However, no studies have been conducted on organochlorine pesticides residues in water and histopathological alteration in fish at Klong 7.

Histological technique is a useful tool for the observation of basic histology and pathological lesions occurring in the metabolic organ after sublethal exposure to the pesticide in environment. Moreover, gas chromatography is a suitable technique for quantitative analysis of the pesticide residue concentrations in the water.

Objectives

1. To assess histopathological alteration occurring in the Siamese glassfish *Parambassis siamensis* liver at Klong 7 agricultural area, Pathum Thani Province.
2. To determine qualitative and quantitative analysis of organochlorine pesticide residues in water at Klong 7 agricultural area, Pathum Thani Province.
3. To assess effects of endosulfan on liver of the Siamese glassfish *Parambassis siamensis*.

Anticipated benefits

1. Obtain basic knowledge about histology of liver of the Siamese glassfish *Parambassis siamensis*.
2. The toxicological effects of the contaminated water on the Siamese glassfish *Parambassis siamensis* can be used to determine the suitability and safety of using pesticides that affect the environmental quality.

CHAPTER II

LITERATURE REVIEW

Pesticides

Over the centuries, humans have developed many ingenious methods in their attempts to control the invertebrates, vertebrates, and microorganisms that constantly threatened the supply of food and fiber as well as posing a threat to health. So, the use of pesticides plays a necessary and essential role. This practice constitutes an important aspect of modern agriculture as they help to control pests and plant diseases in order to become domestically self sufficient in food production, as well as produce adequate surplus yields for export and to supply the national industries.

History

Although the use of chemicals to combat agricultural pests dates from antiquity, the large-scale utilization of chemicals as major components of pest management systems is a twentieth century development. However, types of chemicals in use have changed substantially in response to environmental concerns that have arisen since their introduction.

As late as 1950, many inorganic chemicals were still in use, including calcium arsenate, copper sulfate, lead arsenate, and sulfur (Klassen et al., 1980) but, with the exception of sulfur, these materials were almost completely displaced by synthetic organic pesticides in subsequent years. The 1940s and 1950s were productive years in terms of new synthetic organic chemistry. In Europe, supplies of traditional botanical insecticides, such as pyrethrum extract and nicotine, essential for crop production, were limited by wartime blockades and shortages (Klassen et al., 1980). The chemical industry faced a major challenge in its efforts to synthesize and manufacture replacements for materials that were critically needed to protect crops from insect pests and people lining in tropical areas from malaria and other insect-borne diseases. The discovery of the insecticidal properties

of hexachlorocyclohexane almost simultaneously in France and England in 1940 (Krieger, 2001) was one of the first successes. The discovery of the insecticidal activity of lindane, followed by the widely acclaimed successes of DDT in controlling vector-borne diseases, reinforced efforts to discover and commercialize new synthetic insecticides.

At the end of World War II, newly developed chemical technologies became the basis for the manufacture of a number of new insecticides, particularly the application of Diels-Alder reaction to synthesize cyclodiene insecticides. Because their acute mammalian toxicity was generally low and their spectrum of activity was very broad, such insecticides could be used to control many agricultural insect pests. The organochlorine insecticides were described as “nerve poisons” (Krieger, 2001). Their mode of action was not clarified until some years after their use had become widespread, but because their mammalian toxicity was generally low and their spectrum of activity was broad, they were widely used in agriculture. In 1947, several N-methylcarbamates that possessed significant acethycholinesterase inhibitory activity were synthesized in Switzerland by the Ciba-Geigy Company (now Novartis) and developed as insecticides. One of the best known of the carbamate insecticides is carbaryl (N-methylnaphthyl carbamate).

The high mammalian toxicity for many organophosphates calls for extreme caution in their practical application, but by varying constituent patterns, many less toxic analogs were manufactured and approved for uses as insecticides, fungicides, and plant growth regulators, among other agricultural uses. They are widely used because their environmental persistence is low and they are highly effective.

These new developments in the control of agricultural insect pests were paralleled in the search for chemical agents for control of weeds and plant diseases. Research in the nature of plant growth regulators led to the identification of indoleacetic acid as the first of the plant growth hormones. Since then many, new herbicides that represent a wide variety of chemical classes have been commercialized to improve environmental safety, selectivity, and weed control at low rates of application. As knowledge of biochemical targets has

increased through studies of metabolism and mode of action of pesticides, screening techniques have been improved to make it possible to identify candidate compounds that are effective at specific receptor sites. The introduction of newer synthetic techniques such as combinatorial chemistry which can generate large numbers of new compounds made it is possible to increase the throughput of compounds.

Organochlorine pesticides

Definition of Pesticides

Pesticides are defined under the Federal Environmental Pesticide Control Act as including; (1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest and (2) any substance or mixture of substances intended for uses as a plant regulator, defoliant, or desiccant (Hayes, 2001). The chlorinated hydrocarbon compounds include such important insecticides as DDT, BHC, chlordane, and dieldrin. All compounds which belong to this group are characterized by (1) the presence of carbon, chlorine, hydrogen, and sometimes oxygen atoms, including a number of C-Cl bonds; (2) the presence of cyclic carbon chains (including benzene rings); (3) lack of any particular active intramolecular sites; (4) apolarity and lipophilicity; and (5) chemical unreactivity (i.e., they are stable in the environment) (Matsumura, 1971).

Several kinds of pesticides have been used over the past decades in the attempt to defeat that huge number of crop-eating insects, approximately 700 species worldwide that caused infective and parasitic diseases to humans and loss to harvest. The use of pesticides has contributed to the drastic reduction in those diseases transmitted by insects, most of them life-threatening, while protecting crops during growth and storage. Before World War II the selection of insecticides was more or less the same as those available for a thousand and more years before. It was in the 1940s and '50s that a new concept of pest control took hold, opening a new era of synthetic, highly effective compounds (Nollet, 2000).

The extensive use of synthetic pesticides was at first greeted with enthusiasm, but in a few years it appeared clearly that these compounds and their residues contaminate ground and surface water. In most cases these substances were organochlorinated compounds, also known as chlorinated hydrocarbons, chlorinated organics, chlorinated insecticides, chlorinated synthetics, or organochlorinated pesticides (OCPs) (Table 2-1). Their use has been superseded in most countries, but interest in these compounds is still high. In several organisms, organochlorines are found in a higher concentration than in the environment in which they live, because the living organisms have accumulated of pesticides according of the food chain.

Table 2-1 Organochlorine pesticide status by the announcement no. 2, Toxic and Substance Act, the Ministry of Industry, Thailand (Chulin, 2002).

Common Name	Formula	Molecular Weight	Banned Year
Aldrin	$C_{12}H_8C_{16}$	364.9	1988
Dieldrin	$C_{12}H_8C_{16}O$	377.9	1988
o, p'-DDE	$C_{14}H_8C_{14}$	315.9	-*
p, p'-DDE	$C_{14}H_8C_{14}$	315.9	-*
o, p'-DDD	$C_{14}H_{10}C_{14}$	318.0	2001
p, p'-DDD	$C_{14}H_{10}C_{14}$	318.0	2001
o, p'-DDT	$C_{14}H_9C_{15}$	354.5	1981
p, p'-DDT	$C_{14}H_9C_{15}$	354.5	1981
Endosulfan	$C_9H_6C_{16}O_3S$	406.9	2005
Endosulfan sulfate	$C_9H_6C_{16}O_4S$	422.9	2005
Endrin	$C_{12}H_8C_{16}O$	380.9	1979
Heptachlor	$C_{10}H_5C_{17}$	373.3	1988
Heptachlor epoxide	$C_{10}H_5C_{17}O$	385.8	1988
Hexachlorobenzene (HCB)	C_6H_{16}	284.8	2001
Methoxychlor	$C_{16}H_{15}C_{13}O_2$	345.7	Available

* Non recorded

Physical and chemical properties

The popularity of chlorinated pesticides was based on certain important properties: extremely high stability, very low solubility in water, high solubility in organic media, and high toxicity to insects, but low toxicity to humans

a. Dichlorodiphenyltrichloroethane (DDT)

Technical DDT is a white to cream-colored amorphous waxy powder, and pure DDT is a crystalline powder. The melting point of pure compound is 109°C, and its setting point (crystallization on slow cooling) is between 103° and 105°C. DDT is one of the most apolar compounds known to exist; hence it is soluble in most apolar organic solvents and is practically insoluble in water and cold ethanol. Its water solubility is less than 2 ppb. The average commercial product contains about 70% of the pure p, p'-DDT. Increasing the amount of chloral used in DDT preparation increases the purity of the product. The major contaminant of crude DDT is o, p-DDT, which is not as insecticidal as p, p'-DDT. DDT is very stable. Solid-from DDT is not decomposed by sunlight. Pure DDT is stable to the action of heat and doses not decompose below 195°C. The toxicity of DDT to man is rather accurately known. A single dose of 10 mg/kg produces illness in some but not all people, and convulsions frequently occur at a dose of 16 mg/kg. Dose as high as 285 mg/kg have been taken without a fatal result. The oral LD_{50} of DDT for rats is 250 mg/kg (Matsumura, 1976).

The most notorious pesticide ever used is by far DDT. It was synthesized in 1874 by a German student but was rediscovered by Paul Mueller, a Swiss scientist who recognized it as a powerful insecticide for the control of some important loused borne diseases. After World War II, DDT was used in agriculture for a pest control. The results were excellent: DDT proved itself very effective against a number of pests, and agricultural yields increased rapidly (Nollet et al., 2000).

b. Dichlorodiphenyldichloroethane (DDD) and Dichlorodiphenylchloroethane (DDE)

DDT is very stable, chemically as well as biochemically, except in the presence of alcoholic alkali which dehydrochlorinates it to form DDE which is nontoxic to insects (Matsumura, 1976). The compound DDE is a metabolite of DDT as well as an impurity (Nollet et al., 2000). Pure DDD is a white crystalline solid with melting point of 109°C, and the technical product has a setting point of 86°C. It has a sweet odor. Its solubility is similar to that of DDT, but it is dehydrochlorinated in alkali as a slower rate than DDT. In general, DDD is less effective than DDT for controlling insects, with some exceptions. It is superior to DDT for controlling black fly larvae, red-banded and fruit tree leafrollers, tomato and tobacco hornworms, and the Mexican bean beetle. It is 1/5 to 1/10 as toxic as DDT to mammals, with an oral LD_{50} in rats of 3,400 mg/kg (Matsumura, 1976).

The compound DDD was used for controlling a number of insects on vegetables and tobacco. It is also very effective against mosquito larvae. It can be released to the environment through its use and as a biodegradation product of DDT. It binds very strongly to soil in a dose that not reach appreciably to groundwater, although it can be transported there. When in water, it adsorbs by sediment (Nollet et al., 2000).

c. Methoxychlor

Another important DDT analog is methoxychlor (DMDT, or methoxy-DDT). Methoxychlor is a white crystalline solid with a melting point of 89°C. Technical methoxychlor is a pale buff to gray flaky powder and has a setting point of 69°C. The technical product contains about 89% of the pure p, p'-isomer with the remainder mostly the o, p-isomer. The water solubility is quoted as 0.1 ppm and it is soluble in most organic solvent and subject to dehydrochlorination. Methoxychlor is only 1/25 to 1/50 as toxic as DDT to mammals. It has an LD_{50} in rats of 6,000 mg/kg which makes it essentially nontoxic (Matsumura, 1976). Methoxychlor is rarely phytotoxic and is used on crops including several types of seeds. It is also effective against mosquito larvae and houseflies. It is also used as an insecticide on cattle (Nollet et al., 2000).

d. Cyclodienes

After World War II, cyclodienes, a new class of pesticides, appeared on the scene, comprising aldrin (1948), dieldrin (1948), heptachlor (1949), endrin (1951), endosulfan (1956), hexachlorpbenzene (HCB), and some other of minor importance. Most of them are very stable in sunlight and persistent in soil, and they were used to control termites and other insects (Nollet et al., 2000).

1. Endrin: The endo-endo isomer of aldrin is isodrin which like aldrin is converted to its epoxide endrin which in turn is the endo-endo isomer of dieldrin. Isodrin and endrin are less stable than their endo-endo isomers; and their toxic effects are similar to those of aldrin and dieldrin. Technical endrin is a light tan powder while the pure compound is a white crystalline solid with a melting point above 200°C. Chemically, it is very similar to dieldrin, but endrin can be easily degraded by heat and light. Endrin is highly toxic with an acute oral LD_{50} in rats of 11 mg/kg for the pure compound (Matsumura, 1976). Endrin is produced and sold since 1986. It has been widely used as an insecticide on cotton as well as an avicide and rodenticide (Nollet et al., 2000).

2. Aldrin: A white crystalline solid with a melting point of 100-103°C is a residual compound with a vapor pressure of 6×10^{-6} mmHg at 25°C. Aldrin is almost insoluble in water (0.2 ppm) and soluble in most organic solvents. Aldrin is stable to alkali and dilute acid. It is readily converted in plants and animal tissues and in the soil to its epoxide dieldrin. Hence, aldrin shows the same toxic effects as dieldrin, and both have very similar oral LD_{50} for rats in the range of 55-60 mg/kg (Matsumara, 1976). Aldrin is a pesticide widely used in agriculture, to control insects in soil, and also in public health for defeating mosquitoes and tsetse flies (Nollet et al., 2000).

3. Dieldrin: epoxy of aldrin is one of the most persistent chemicals ever known. It has been used extensively since 1952, especially in situations where a long-lasting residual effect is advantageous. Dieldrin has a melting point of 173°C. The pure compound is an odorless white crystalline solid. Dieldrin is as insoluble as aldrin in water (0.25 ppm) and less soluble inorganic solvents. Only such procedures as treatments with strong acid or long exposure to intense ultraviolet light are known to decompose dieldrin. It is less apolar than aldrin. Dieldrin can be absorbed through the skin and has an acute oral LD_{50} in rats of 60 mg/kg. It acts as a stimulant of the central nervous system (Matsumara, 1976).

4. Heptachlor and Heptachlor epoxide: Heptachlor is derivatives of Chlordane. Heptachlor, a white crystalline solid, is four to five times more insecticidal activity than technical chlordane. The acute oral LD_{50} of heptachlor for rat is 90 mg/kg. Heptachlor is stable to heat up to 160°C, light, moisture, air, acids, bases, and oxidizing agents. In biological systems, heptachlor is converted to its epoxide and is stored in that form. Heptachlor epoxide is more toxic than heptachlor, so the epoxidation is a vital process to produce toxicity (Matsumura, 1976). Heptachlor is a chemical used extensively as a termiticide in homes and buildings and as a pesticide on food crops, especially corn. The use of heptachlor has been regulated, and it is now used only for control of fire ants in power transformers. Heptachlor epoxide is not commercially available but is a result of heptachlor degradation.

5. Endosulfan and Endosulfan sulfate: A related insecticide, also an acaricide, is endosulfan. Two other cyclodienes used to control insects on crops are endosulfan, a mixture of the two isomeric forms endosulfan I and endosulfan II, and endosulfan sulfate. Endosulfan is a mixture of two isomers, one with a melting point of 106°C and the other 212°C. It is

moderately soluble in most organic solvents and insoluble in water. Endosulfan is absorbed through the skin and has an acute oral LD50 in rats of 30-79 mg/kg. This compound caused a large-scale fish kill in the Rhine River by accidental spilling (Matsumura, 1976).

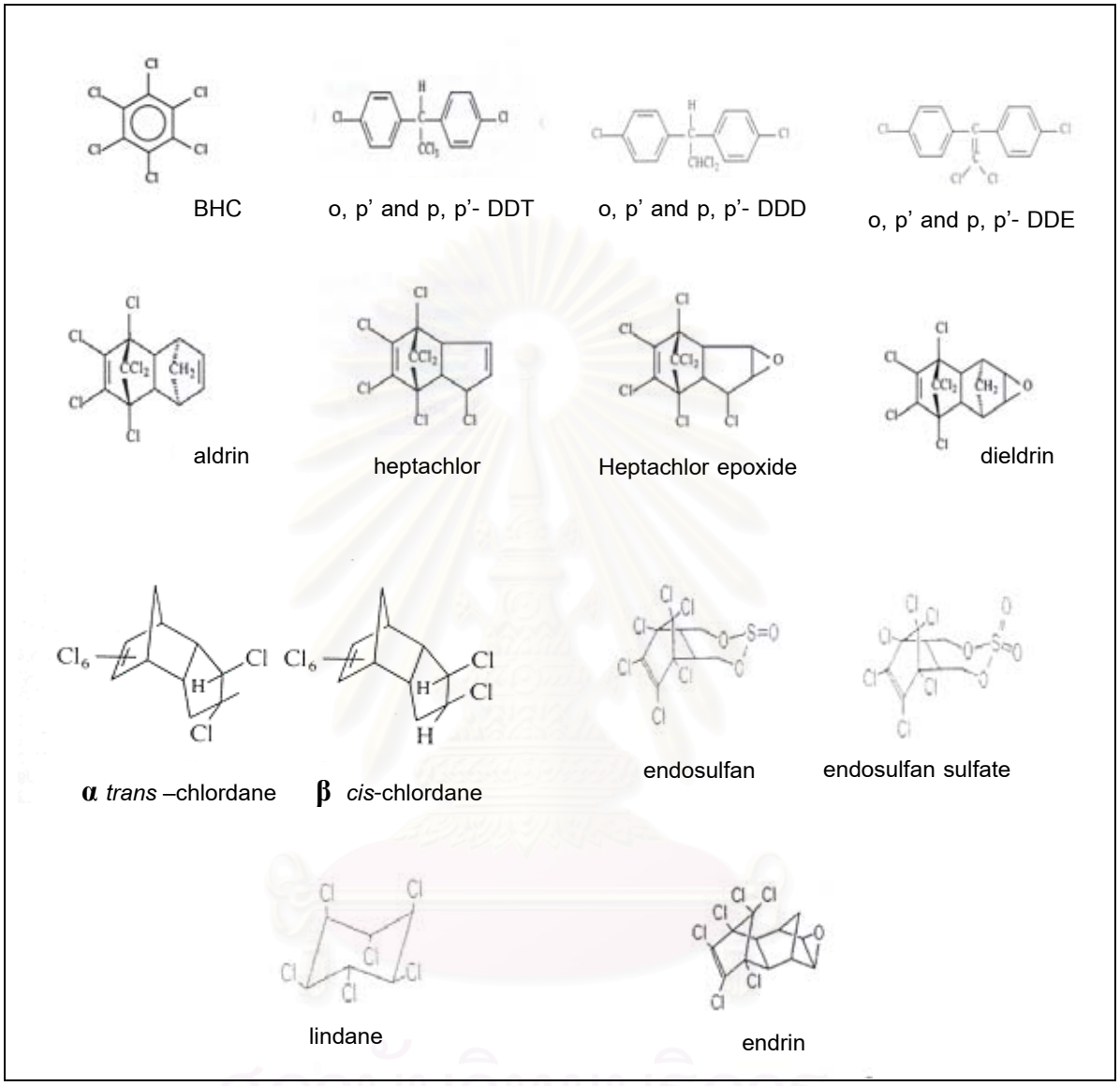
e. Hexachlorobenzene (HCB) or benzene hexachlorine (BHC)

BHC is another OCP used until 1965 to protect crops against fungi. It is high toxicity. BHC was first prepared in 1825 by Michael Faraday. It is a grayish or brown amorphous solid with a characteristic musty odor. Crude BHC begins to melt at 65^0C. It is toxic to mammals with LD_{50} in rats of 125 mg/kg (Matsumura, 1976). BHC is a very stable compound and consequently, is highly persistent in the environment. When released in soil it adsorbs strongly and does not biodegrade, and it is unlikely to contaminate groundwater. BHC interferes with lipid metabolism and transport in living organisms, and it affects liver enzymes. It may affect the central nervous system, dermal irritation, liver, and kidney damage. Thyroid enlargement anemia and pulmonary and stomach damage have also been reported (Nollet, 2000).

Metabolism of some organochlorine pesticides

Mastsumura (1976) reported that DDT degradation *in vitro* in the intestinal contents of Atlantic salmon which found DDE and DDD. Aldrin is converted to its epoxide analog, dieldrin, by mammals, soil microorganisms, plants, and insects. Heptachlor is metabolized to heptachlor epoxide by mammals, insects, plants, and soil microorganisms. Endosulfan sulphate is metabolized from endosulfan by insects, plants, mammals, and soil microorganisms.

Figure 2-1 Chemical structure of some organochlorine pesticides (Matsumura, 1976).



Environmental ecotoxicology

Ecotoxicological effects may be considered through several different organizational levels. The first step consists of the introduction of a toxicant or pollutant into the system. This system may result in biochemical changes at the molecular level. As a result, physiological changes may occur in tissues and organs. These toxicants may result in detrimental alterations of organisms.

Contaminations of pesticide

Persistent organic toxicants are arguably the pollutants of most concern with respect to ecotoxicology because of their abilities to bioaccumulate in lipid (fat) tissue and to become increasingly concentrated in such tissue as they progress through food chains (Manahan, 2003). Many researchers were reported as follows: Zapata et al. (2000) studied environmental pollutants in sediments obtained from Bahic de Chetumal by intraperitoneally injected in nile tilapia (*Oreochromis niloticus*) with sediment extracts which sediment samples for study contained of organic chemicals such as organochlorine pesticides, PCBs, and polynuclear aromatic hydrocarbons (PAHs). In the result, blood examination showed cell degeneration and binucleated leukocytes with abnormal chromatin and the basement of gill lamellae showed foci of hyperplasia, hypertrophy, and edema in gills and liver necrosis.

Tate and Heiny (1996) reported about there collected bed sediment and fish tissue samples in the South Platte River Basin to determine the occurrence and distribution of OC compounds. They found OC concentrations in bed sediment and fish tissues which were related to land-use settings.

In the background, amounts of pesticide residues in aquatic environment have been reported. For example, in Thailand, Tangtrongkijwong (1991) reported about pesticide residues in water and sediment from 25 subwatersheds of the five watershed classes among the four lower southern provinces namely Phattalung, Satun, Songkhla, and Trang. The samples were collected every two months from November 1987 to September 1988;

and the results showed that pesticides found in water samples were aldrin, dieldrin, o, p'-DDT, p, p'-DDT, o, p'-DDE, p, p'-DDE, o, p'-TDE, and o, p'-TDE. Dieldrin was of the highest concentrations which were 1.37, 1.34, 1.89, 2.82, and 2.28 ppb for the first to fifth watershed classes, respectively. Also, he found that concentrations of dieldrin and total DDT were very high in rainy season, but low in cold season and hot season. Senglai (1998) reported that the water and sediment from 13 sites in the Outer Songkhla Lake, Songkhla Province during November and December 1995 (wet season) and March and April 1996 (dry season), found γ-HCH and p, p'-DDT, dominant in OCP residues, were 1,796 and 1,368 times higher than those water samples, respectively. Total OCP residues ranged from < 2-44.5 (x = 12.6) ng/l in water, < 0.01-282.7 (x = 22.2) mg/kg in sediment.

Klabrod (1998) reported that the OPP residues in agricultural soils of Songkhla Province, Thailand. The samples were collected 3 agrosystems i.e. vegetable 5 sites, orchard 3 sites, and 2 rubber plantation areas. The soil samples were collected during different seasons in 1998 i.e. February, June, October, and December; and the results showed that four OPPs were found in the range of monocroptophos 0.43-6.55 ppb, dimethaoate 0.49-12.00 ppb, methyl-parathion 0.39-174.16 ppb, and malathion 0.06-30.75 ppb.

Tayapatch et al., (1994) reported that qualitative and quantitative analyses of organochlorine insecticide residues were conducted in water, sediment, aquatic plants and animals from 3 freshwater reservoirs: Bueng Boraphed, Nakhon Sawan; Nonghan, Sakon Nakhon and Kwanphayao, Phayao during March-September 1989. The results indicated that 5 kinds of insecticides were found in most samples such as lindane, heptachlor, aldrin, dieldrin, DDT, and derivatives. Dieldrin was detected at higher concentration and found in all samples. The residue level of dieldrin ranged from <0.01-0.12 ppb in water, 0.005-0.036 ppm in sediment, <0.001-0.138 ppm in aqutic plants, and <0.001-0.037 ppm in aquatic animals, respectively.

Sources of toxicants

Sources of toxicants that organisms acquire from their surroundings may be divided into three general categories: toxicants are ingested with food or water into the alimentary tract, toxicants are absorbed from ambient surroundings, especially by fish that live in water.

Some pesticides such as organophosphorus pesticides have generally low persistence in the environment, but they may persist in water and accumulate in certain aquatic vertebrates. Hall and Kolbe (1980) reported that frogs were resistant to cholinesterase inhibitors thus; it was suspected that they might accumulate the pesticides. Tadpole's concentrated pesticides from water up to 60 times. Those tadpole exposed to 1 ppm parathion and 5 ppm fenthion were lethal when they were fed to mallard ducks. Metabolites of parathion and fenthion produced by the tadpoles were rapidly excreted and it was concluded that they played a small role in the toxicity of the larvae to ducks. Dangerous levels of some pesticides might be accumulated by amphibians in nature and might adversely affect carnivorous species.

John and Prakash (2003) brought the freshwater teleost fish *Mystus vittatus* exposed to metasystox (OP) and carbaryl (carbamate) separately for 30 days. The concentrations of pesticides were 4 ppm and 7 ppm for metasystox and carbaryl, respectively. The result found that the accumulation of metasystox was more as compared to carbaryl. The general trend in the residue levels were found to be muscle > gill > blood > kidney in both cases.

Ueno et al. (2002) determined concentrations of persistent OCs such as PCBs, DDTs, chlordanes (CHLs), HCH, and HCB in the liver of blue fin tuna (*Thunnus thynnus*) collected from Japanese coastal waters. This fish species was a biomonitor for pollution in the open sea ecosystem. The levels of these chemicals did not show correlation with body length. These results suggested significance of dietary uptake of PCBs, DDTs, and CHLs compared to the intake via the gill.

Sapozhnikova et al. (2004) studied on the determination of OC and OP pesticides in sediments and fish tissues; and evaluate the relative ecological risk of these compounds. Fish tissues (*Tilapia mossambique* and *Cynoscion xanthula*) were collected in May 2001 in the Salton Sea. They found ∑DDT in sediments ranged from 10-40 ng/g. DDE concentrations in fish muscle tissues were above the 50 ng/g concentration threshold for the protection of predatory birds.

Kumblad et al. (2000) studied about the concentrations of p, p'-DDT, -DDE, and –DDD in fish of four species (*Scatophagus argus*, *Protosus canius*, *Channa striata,* and *Zonichthys nigrofasciata*) from the large, brackish Songkhla Lake and the Gulf of Thailand. The results showed that the mean ∑DDT concentrations were 33 to 170 ng/g lipid wt. Although DDT for agriculture use was banned in 1983, residue levels can still be recorded in lake indicating present usage on agricultural lands around the lake.

Kunisue et al. (2003) determined DDTs in the resident and migratory birds which were collected from India, Japan, Philippines, Russia (Lake Baikal), and Vietnam. They found that migratory birds from Philippines and Vietnam retained mostly the highest concentrations of DDTs among the organochlorines analyzed, indicating the presence of stopover and breeding grounds of those birds in China and Russia.

Residues in human

In human, there were many studies about residues of pesticides such as; in Japanese was estimated daily intakes of organochlorine pesticides from foods, fish products, meat, eggs, milk, and milk products were analyzed as major sources of these pesticides in the diet. Estimated daily intakes (EDIs) per person were 0.56 mg for total HCH, 0.20 mg for γ-HCH, 0.09 mg for dieldrin, 1.42 mg for total DDT, and 0.15 mg for HCB. Similarly, daily intakes of organophosphorus pesticides, malathion, and chlorpyrifos methyl were 0.22 and 0.24 mg, respectively. Dialy intakes of total HCH, dieldrin and total DDT in fiscal year 1992-1993 decreased 29, 44 and 40%, respectively (Robbin et al., 2004).

Konishi et al. (2001) reported the survey of chlorinated organic compound, DDT, β-BHC, and PCBs which found an increasing contamination in breast milk of lactating women living in Japan. In Thailand, there was reported that found residue pesticides in blood of mothers and newborn babies (63% of total samples) such as α-BHC, γ-BHC, heptachlor, heptachlor epoxide, p, p'-DDE, o, p'-DDT, dieldrin, and o, p'-DDE about 0.16-75.02 ppb (Punviriyapong and Tayapach, 1998).

Toxicants may enter organisms by way of food. In some cases, residues of pesticides or other toxic organic compounds applied to food crops may be ingested by animals or even humans. In nature, such toxicants may enter food chains as the organisms n the higher trophic levels in the food chains consume the other organisms in the lower trophic levels in the food chains. Since OC pesticides, such as DDT, are known to spread far and wide because of their resistance to decomposition and their lipophilic properties; the global monitoring of these pesticides has become one of the world's most important priorities.

Table 2-2 Drinking Water Standards recommended limits for selected pesticides by environmental protection agency (E.P.A.) (Chulin, 2002).

Type of Pesticide	Maximum Allowable Concentration (μg/L)
aldrin	0.1
BHC	1.0
chlordane	3.0
DDT	1.0
diedrin	0.1
endosulfan & derivatives	3.0
endrin	0.5
heptachlor	0.018
heptachlor epoxide	0.2
lindane	2.0
methoxychlor	0.035
toxaphene	0.005
malathion	7
carbaryl	60

Toxicology

Toxicology is the study of the adverse effects of chemicals on living organisms. The variety of potential adverse effects and the diversity of chemicals present in our environment in combination make toxicology a very broad science. Toxicologist usually divided the study. Types of exposure of animals to chemicals are divided into four categories: acute, subacute, subchronic, and chronic. The principle goals of the subchronic study are to establish a non-observable effect level and to further identify and characterize the specific organ(s) affected by the test compound after repeated exposure (Klaassen and Eaaton, 1991).

Effects on cell and chemical of cell

Effects of contaminants on living organisms are fundamentally biochemical in malfunctions in cellular metabolic processes and eventually in morphological /physiological changes (Couch and Fournie, 1993). Cells are active participants in their environment, constantly adjusting structure and function to accommodate changing demands and extracellular stresses. Cells maintain normal homeostasis such as cells encounter physiologic stresses or pathologic stimuli, they can undergo adaptation and achieving a new steady state and preserving viability. The principal adaptive responses are atrophy, hypertrophy, hyperplasia, and metaplasia. If the adaptive capability is exceeded, cell injury develops. Within certain limits, injury is reversible, and cells return to a stable baseline; however, with severe or persistent stress, irreversible injury results, and the affected cells die.

Two principal patterns of cell death are recognized; these have distinct mechanisms, but there is also considerable overlap between the two processes:

1. Necrosis occurs after an exposure to toxins and is characterized by cellular swelling, protein denaturation, and organellar breakdown. This pathway of cell death may result in considerable tissue dysfunction.

2. Apoptosis occurs as a result of an internally controlled 'suicide' program, after which the dead cell are removed with minimal disruption of the surrounding tissues. This occurs in physiologic states when cells receive a variety of pathologic states (Robbin et al., 2004).

Table 2-3 Summarizes some of the important differences between necrosis and apoptosis (University of Florida PA Program Fall, 2004).

Type	Necrosis	Apoptosis
Stimuli	Pathologic (hypoxia, toxin, etc.). Consequence of irreversible cell injury. Think of necrosis as "cell homicide".	A physiologic, genetically regulated process. Occasionally activated by pathologic stimuli. Think of apoptosis as "cell suicide".
Histology	-Typically large numbers of cells affected -Cell swelling -Cellular acidosis -Organelle disruption -Loss of membrane integrity -Coagulation or liquefaction of cell protein.	-Usually only a few cells affected. -Cell shrinkage due to hydrolysis and cross-linking of structural proteins within the cytoplasm and nucleus. -Organelles remain normal. -Cell breaks down into membrane-bound fragments (apoptotic bodies) which are taken up by neighboring cells.
DNA Breakdown	Karyorrhexis and karyolysis: random, diffuse fragmentation and dissolution of the nucleus.	Orderly nuclear condensation and fragmentation.
Tissue Reaction	Inflammation with secondary injury to surrounding normal tissues.	No inflammation or secondary tissue injury.
Animation	Necrosis	Apoptosis

Some pesticide, DDT has been used for pest control in both bio-accumulated and persistent in the soil environment. DDT or its metabolites have been found in surface water, groundwater, soil, and dairy products. Another pesticide, malathion, is a broad spectrum

pesticide, it has been detected in groundwater at concentration of up to 6.2 µg/l in several well in California, Missouri and Virginia. This water is used for drinking purpose. Since prior to distribution, water is treated with various reactive chemical including chlorine and ozone. An additional concern and one rarely investigated is whether the products formed during this treatment render the pollutants more or less toxic (Robbin et al., 2004).

Masten et al. (2001) reported that used DDT and malathion on gap junction intercellular communication (GJIC) were determined using a rat liver epithelial cell line. These results suggested that ozone could effectively remove malathion from solution without forming GJIC-toxic products, but was less effective in eliminating DDT from solution.

Xuanhui et al. (2003) reported that low concentration (0.25 $mg.L^{-1}$) monocrotophos (an OP pesticide) affected to an increasing of Na^{+}/K^{+}- ATPase activity in the chloride cells of branchiae proliferated. The damage in gill under sublethal monocrotophos exposure included hypertrophy and edema, and thus, the lamellar epithelium became thick and lifting, and the lamellae were bulbing or fusing. The ultramicrostructural changes of the gill of the exposure fish were the swollen and even rupture of r-ER, mitochondria, microtubule and nuclear membrane.

Wang et al. (2003) reported that 5 OP insecticides (dichlorvos, methamidophos, monocrotophos, acephate, and trichlorfon) induced chromosome aberration in a Chinese hamster ovary (CHO) cell line. And induction potential was dichlorvos> methamidophos> monocrotophos> acephate> trichlorfon. So, contamination of the environment by pesticides on the other hand has created many adverse effects to human health.

Patil et al. (1990) reported that the metabolic modulations in liver of the fish, *Boleophthalmus dussumieri* exposed to 2 and 4 ppm of an OP, monocrotophos up to 7 days were studied. The result showed that fish showed low protein levels in the liver but liver showed considersble increase in lipid levels than the control. Jyoti et al. (1989) had been experiments on a freshwater fish, *Channa punctatus* by using 96 h. LC_{50} value of an OP

pesticide, malathion. A significant fall in protein and RNA contents was observed in all the studied organs. As Sancho et al. (1997) found that fenitrothion (0.2 mg/L.) induced proteins decreased in liver of the European ell (*Anguilla angguilla*).

Effects to enzymatic activities

This topic reported some previous researchers had observed the effects of different pesticides on enzymatic activities of non-target species of an ecosystem. Pesticides affected all members of an ecosystem, from the smallest invertebrates to birds and humans. Most toxic pesticides in both urban and agricultural settings were responsible for the deaths of many birds, fish, and smaller aquatic animals that fish depended on for food. Amphibians lived near water reservoirs or in agriculture were another important component of the food chain.

Silva et al. (1993) observed the effects of sublethal doses (500mg/kg) of folidol 600 (OP). It inhibited 90% of plasma cholinesterase activity after 4 h from the initial time and changed many behaviors in the first 96 h i.e. lowering of motility with loss of equilibrium and frequent unnatural body positions, uncoordinated movements leading to incapacity of food in take, and increase in the levels of respiratory frequency. These behavioral changes cause by OP effect as inhibitors of cholinesterase by OP blocking of the repolarization of postsynaptic membrane at the neuromuscular junctions, leading to functional and behavioral changes (Palawski et al., 1983).

Swann et al. (1996) reported that chlorpyrifos (OP insecticides) inhibited acetylcholinesterase, the acetylcholine innervated ciliated epithelial cultures of frog palate, which atropine only blocked the initial stimulatory response with chlorpyrifos. In addition, the ingredients compounds caused mitochondrial damage, including swelling, disruption of cristae, and loss of matrix. Stocks of AChE in fishes were usually greater than in other animals, and the lower amounts of AChE were needed to sustain life. Therefore, higher

levels of AChE inhibition could be expected before death occurs in fishes (Aguiar et al., 2004).

Garcia et al. (2000) reported that p, p'-DDT affected to an increasing of hepatic cytochrome p450 content and glutathione s-transferase activity in liver tissue of two Mediterranean mesopelagic fish species; the benthic *Lepidorhombus boscii* and the demersal *Phycis blennoides*. Khan et al. (2003) reported the effects of pesticides (cypermethrin and permethrin) reduced cholinesterase activity and the total protein both in the kidney and liver. Total protein content also decreased in non-target vertebrate fauna after pesticide treatment, indicating pesticide-produced changes in the biochemical systems of non-target organism. So, pesticides disturbed protein synthesis.

Aguiar et al. (2004) studied the effect of folidol 600 (methyl parathion) on fish (*Brycon cephalus*). The result showed that liver alanin aminotransferase (ALAT) decreased 59.4%, indicate tissue damages, and the increase of heart and plasma aspartate aminotrasferase (AAT) suggested tissue injury. Moreover, hepatic glycogen and glucose levels decreased 80.4% and 55%, respectively. Gruber and Munn (1998) observed the effects of OP and carbamate insecticides in agricultural water on Carp (*Cyprinus carpio*), which indicated that mean whole-brain ChE activity was 34.2% less than that of carp from control. The depressed ChE activity was in response to ChE-inhibiting insecticides. Although OP and carbamate insecticides can break down rapidly in the environment, this study suggested that in agricultural regions where insecticides were applied for extended periods of the year, non target aquatic biota might be exposed to high levels of ChE-inhibiting insecticides for a period of several months.

Sohn et al. (2004) studied the toxic mechanism of endosulfan (an OC pesticide) on *Saccharomyces cerevislae* and human cell lines, which demonstrated by increased thiobarbituric acid-reactive substance (TBARS) production. The results suggested that endosulfan-induced inhibition of yeast growth and cytotoxicity in human cell lines were closely associated with endosulfan-induced reactive oxygen species (ROS) generation and

respiratory inhibition. Endosulfan is a chlorinated cyclodiene pesticide, and like other members of this chemical group, the predominant toxicological effect is over stimulation of the central nervous system (by inhibiting Ca^{2+}, Mg^{2+}-ATPase and antagonizing chloride ion transport in GABA (gammaaminobutyric acid) (Paquette and Liem, 1999).

Gul et al. (2004) collected freshwater fish (Cyprinidae) from polluted, which glutathione-s-transferase (GST), lacticdehydrogenase (G6PDH), superoxide dismutase (SOD) and molondialdehyde (MAD) affected to molecules having unpaired electrons, usually derived from oxygen and its various reactive intermediates, but also derived from metabolic reactions (Liang et al., 1992). Generation of oxyradicals is thought to be involved in the aetiology of many human diseases and is likely to be the same for fish. When an oxidative stress took place, most of the superoxide anions (O_2^-) released in the cell were transformed into hydrogen peroxide (H_2O_2) molecules produced inside the peroxisomes, and were destroyed exclusively by catalase (CAT) (Babo and Vaseur, 1992). Superoxide dismutase (SOD), as well as catalase (CAT), was an inducible enzyme that reacted with activated oxygen species.

One of the most frequently used biomarkers indicating the overall lipid peroxidation levels was the plasma concentration of malondialdehyde (MDA), one of the several by products of lipid peroxidation. G6PDH was the key enzyme of pentose phosphate pathway. NADPH produced by G6PDH was an important cofactor in biosynthesis of lipids. A high level of NADPH increased biosynthesis of lipids in liver. LDH was a glycolitic enzyme. Primary damages occured in hypoxic conditions as a result of tissue damages in liver by the effects of xenobiotics (Gul et al., 2004). Fish from the contaminated area displayed a tendency toward decreased GST activity. This might be related to the fact that xenobiotics were detoxificated by GST pathways, which thus enabled fish exposed to toxic pollutants to survive (Chatterjee and Bhattacharya, 1984).

Effects to immunology and haematology

Effects of sublethal and chronic pollutants on the immune responses of fish when received trichlorfon (OP) showed a leucopenia and decrease in the percentage of PMN cell and decrease in myeloperoxidase activity in neutrophils and in lysozyme levels in serum (Siwicki and Studnicks, 1994). Trichlorfon depressed the nonspecific immune response pf carp: a decreased in the phagocyte activity of neutrophils and macrophages, as well as decreased in lysozyme activity (Cossarini et al., 1991).

Zabihi et al. (2004) reported diazinon (DZN), an OP pesticide, was examined to the immunotoxic effects to mice. The results showed that DZN at 25 mg/kg could suppress both humeral and cellular activity of the immune system. Areechon and Plumb (1990) reported the effect of a sublethal concentration of malathion to the hematology on Channel catfish (*Ictalurus punctatus*), which shown an increasing hematological components to fish erythrocytes and decreasing leukocytes. Dutta et al. (1992) studied that malathion induced changes in haematological parameters of an Indian catfish *Heteropneustes fossilis* (Bloch), which showed a decrease in the number of red blood cell and haemoglobin content in tested fish.

Sampath et al. (1993) found the decreasing in the erythrocyte count, heamoglobin concentration and mean corpuscular volume (MCV), leading to microcytic anaemia in *Oreochromis mossambicus* exposed to sublethal levels of Ekaluk, an OP pesticide.

Effects to reproductive system and DNA damage

Tolerance to stress might be lower in the reproductive tract than in many other organ systems (Couch and Fournie, 1993). Population declined from reproductive impairment was potentially the most serious biological impact of a toxicant-compromised environment. Reproductive toxicity could be defined as a dysfunction of the reproductive system induced by chemical agents and includes effects on any of the processes. The animal effects of

drugs and environmental chemicals on the animal reproductive system were of major health concern, and incidents of chemically induced germ-cell damage and sterility appeared to be on the increase (Hodgon, 1997).

Some pesticide can affect to embryonic development of mouse or sea urchin, such as methoxychor, dieldrin, and lindane. Dieldrin is an environmental estrogen that can affect ovarian function and the insecticide lindane affect early development of zebrafish, rabbit, bovin and mouse embryos. These pesticides could also affect on sperm of various species. Lindane altered the acrosome reaction of human sperm. Dieldrin and methoxychor reduced the fertilization efficiency of sea urchin sperm (Dinnel et al., 1989). Pesaudo et al. (2004) studied the effects of methoxychlor, dieldrin, and lindane on fertilization and early development of sea urchin egg. The result showed the rate of fertilization decreased, increased the rate in polyspermy, delayed or blocked the first mitotic divisions, and altered early embryonic development.

In birds, adverse effects of pesticide included: reproductive failure due to egg-shell thinning, high embryo mortality, malformations in chicks, abnormal reproductive behavior, immunotoxicity and teratogenesis. Bird eggs were highly susceptible to OC contamination and, therefore, provide some indication of contaminant levels in the mother. OC compounds were conveyed from the mother to the eggs via lipids in varying quantities, depending on the species (Aurigi et al., 2000). Another effect was produced by chlorinated hydrocarbons, such as DDE and dieldrin, were thinning of the eggshells in different of birds. The egg shell was formed by the mucosal cells of shell gland. An effect of pollutant on the shell was therefore exerted either directly, on the function of the mucosal cells, or more indirectly, on regulatory factors of the activity of these cells. This shell thinning would lead to cracked or broken eggs and other deleterious consequences for reproduction, effects which had resulted in near extinction of the populations of several species of birds (Lundholm, 1987).

Effects to histopathology

Use of the histopathological approach in toxicological pathology of animals has a number of strengths. A number of important tissues may be simultaneously studied while maintaining in situ cellular, tissue, and organ system relationships. Maintenance of spatial relationships is required to appreciate biological effects associated with toxicity in localized portions of an organ and the subsequent derangement (s) in fluids, tissues, or cells at other locations (Couch and Fournie, 1993).

Stentiford et al. (2003) studied on the histopathological alterations in selected organs and tissues of three species of estuarine fish (*Platichthys flesus*, *Pomatoschistus minutus,* and *Zoarces viviparus*). The liver of fish showed histopathological alterations such as hepatocellular fibrillar inclusions, neoplastic toxicopathic lesions, hepatic foci, and hepatocellular adenoma. However, it is known that the OP used for the disease treatments can also cause histopathologic changes in many fish organs (*Prochilodus linneatus*). Veiga et al. (2002) reported that Trichlorfon (OP) pesticide alternated kidney histology tested (0.2 µm/l), showing glomerular atrophy, hypertrophy of the kidney tube cell, and blood overflowing from capillaries with pyknotic nuclei.

Furthermore, some OP pesticides such as monocrotophos, sublethal doses effected to gill lesions in perch (*Anabus testudineus*). Histopathological changes, degeneration and necrosis of epithelial cells were found very prominent and also found disruption of epithelia cells from pillar cells (Santhakumar et al., 2001). Moreover, malathion (OP) pesticides at sublethal concentrations (0.01 and 0.02 mg/L) for 10, 20, and 30 days changed histological gills of fish (*Gambusia affinis*). The gill lesions included necrosis and desquamation of secondary lamellar epithelium, lifting up to epithelium, intraepithelial edema, fusion of adjacent 2nd lamellae, and hypertrophy and hyperphasia of epithelial cell (Cengiz and Unlu, 2003).

Siamese glassfish *Parambassis siamensis* (Fowler, 1937)

General classification of *P. siamensis* is:

Kingdom Animalia

Phylum Chordata

Subphylum Vertebrata (Craniata)

Superclass Gnathostomata

Class Osteichthyes

Order Perciformes

Suborder Percoidei

Family Chandidae

Genus *Parambassis*

Species *Parambassis siamensis*

Common name: Siamese glassfish

Synonym name: *Ambassis siamensis, Chanda siamensis, Parambassis puntulata, Chanda punctulata,* and *Parambassis punctatus*

Thai name: Pla Kamao, pla pant, and Pla Grajok

The local fishes of this genus are mostly small, whitish or silvery, some of them trans lucent, found in salt, brackish, and fresh waters. Some are strictly fresh water forms, and some occur regularly in both the sea. Its closest relative seems to be *C. ranga* of India, Burma, and Thailand, which has smaller scales and a deeper body. A ventral axillary scale, described as going 2.8 times in the length of the ventral fin. A conspicuous black blotch at the tip of spinous dorsal fin; a dark vertically elongated blotch behind the upper extremity of the gill opening (may be very weak or absent); the second anal spine not much enlarged.

The species of *Chanda* (*Parambassis*) *siamensis* known in Thailand may be distinguished as follow:

- Lateral line continuous

- Scales in lateral line 51 to 53; 4 or 5 rows of scales on cheek; depth 2, and rays III, 13 or 14, and second anal spine shorter than third (Smith, 1965).
- Maximum site at 6.0 cm

They have in common a mainly carnivorous diet such as planktons, insect, and small invertebrate. The fish have occasionally seen in markets and often found in the aquarium trade.

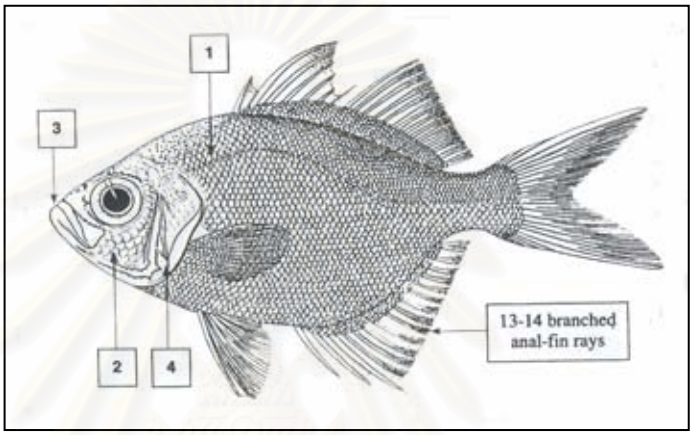


Figure 2-2 Siamese glassfish *Parambassis siamensis* (Rainboth, 1996)

(1) 50 or more tiny scales in lateral series

(2) 3 to 4 rows of scales on check

(3) No teeth on tongue

(4) interopercle non-serrated

A considerable amount of information on the extent of environmental contamination within a given area can be obtained by examining the water bodies there, since these are likely to contain all the contaminants of local importance. However, the levels of these contaminants in the water are continuously changing; therefore these investigations do not always provide a reliable indicator of the general situation. Plants and animals (including fish) that live in water provide a much move reliable source of information on the levels of environmental concentration (Muller and Loyd, 1994). Therefore, fishes are the one of the bioindicators which cannot be ignored.

In many fish species there were many studied about the toxicant of pesticides. For example Siwicki and Studnicka (1994) reported the stimulation of nonspecific immunity after immunosuppression induced by chemical stress in carp (*Cyprinus carpio*). Zepata et al. (2000) reported the effect of environmental pollutants in sediments one Nile tilapia (*Oreochomis niloticus*) from a bay in Mexico.

The biology of fish liver

The liver is large and varies from yellowish brown to dark red in color. The parenchyma is not divided into distinct lobules and is composed of branching, two-cell thick laminae of hepatocytes separated by sinusoids. The appearance of the hepatocytes varies between specimens. The principle difference is the degree of vacuolation in routine preparation resulted from the removal of glycogen and fat during slide preparation. The gross variation in the color of the fresh liver may also be related to difference in glycogen and fat content. The hepatic portal vein branches before entering the posterior side of the liver, and the hepatic vein leaves the anterior side and passes through the transverse septum to the heart. Blood flows from branches of the hepatic portal vein and hepatic artery through the sinusoids to central veins which empty into the hepatic vein. Hepatic arteries are small and contribute little of the blood going to the liver. Pancreatic tissue surrounds branches of the hepatic portal vein within the liver (Grizzle and Rogers, 1976).

The function of fish liver

The liver is a target organ of toxic chemicals because of its blood supply leading to pronounced toxicant exposure and accumulation; its clearance function involving microvasculature, hepatocytes, possibly phagocytic cells, and intrahepatic biliary system; and its pronounced metabolic capacity, critical for internal homeostasis, and for survival of the organism.

The liver serves three main functions: (1) Uptake, metabolism, storage and restribution of nutrients and other endogenous molecules. The liver has a central function in maintaining homeostasis of the organism by synthesis and secretion of molecules into the blood as well as by removal, metabolism, and eventually excretion of compounds. (2) Metabolism of xenobiotics, biotransformation catalyzes the conversion of poorly excretable, lipophilic chemicals into more readily excretable water-soluble compounds. However, during the biotransformation process, many electrophillic reactive species are generated which readily interact with basic cellular constituents such as DNA and protein. This can lead to disruption of normal cellular function and result in toxicity including carcinogenesis. (3) Formation and excretion of bile, bile excretion is important for the elimination of degradation products of endogenous compounds such as heme or steroid hormones; and, in addition, for elimination of xenobiotics, their metabolies, and some metals (Hinton et al., 2001).

The liver detoxification pathways

Inside the liver cells there are sophisticated mechanisms that have evolved over millions of years to break down toxic substances. Every drug, artificial chemical, pesticide and hormone, is broken down (metabolized) by enzyme pathways inside the liver cell. Many of the toxic chemicals that enter the body are fat-soluble, which means they dissolve only in fatty or oily solutions and not in water. This makes them difficult for the body to excrete. Fat soluble chemicals have a high affinity for fat tissues and cell membranes, which are made of fatty substances. In these fatty parts of the body, toxins may be stored for years, being released during times of exercise, stress, or fasting. During the release of these toxins, symptoms such as headaches, poor memory, stomach pain, nausea, fatigue, dizziness, and palpitations may occur (Cabot, 2003).

The body's primary defense against metabolic poisoning is carried out by the liver. The liver has two mechanisms designed to convert fat-soluble chemicals into water soluble

chemicals so that they may then be easily excreted from the body via water fluids such as bile and urine.

The liver detoxifies harmful substances

Basically there are two major detoxification pathways inside the liver cells which are called the phase one and phase two detoxification pathways

a. Phase one: detoxification pathway

An example of the phase one pathway is the cytochrome P-450 mixed function oxidase enzyme pathway. These enzymes reside on the membrane system of the liver cell (called hepatocytes). Animal liver cells posses the genetic code for many isoenzymes of P-450 whose synthesis can be induced upon exposure to specific chemicals. This provides a mechanism of protection from a wide variety of toxic chemical. To put it simply, this pathway converts a toxic chemical into a less harmful chemical. This is achieved by various chemical reactions (such as oxidation, reduction and hydrolysis), and during this process free radicals are produced which, if excessive, can damage the liver cells. Antioxidants (such as vitamin c and e and natural carotenoids) reduce the damage caused by these free radicals. If antioxidants are lacking and toxin exposure is high, toxic chemicals become far more dangerous. Some may be converted from relatively harmless substances into potentially carcinogenic substances. Excessive amounts of toxic chemicals such as pesticides can disrupt the P-450 enzyme system by causing over activity or what is called 'induction' of this pathway. This will result in high levels of damaging free radicals being produced. Substances may cause over activity (or induction) of the P-450 enzymes such as alcohol, dioxin, OP pesticides, and barbiturates (Cabot, 2003).

b. Phase two: detoxification pathway

This is called the conjugation pathway, whereby the liver cells add another substance (eg. cysteine, glucine, and a sulphur molecule) have been caught to a toxic

chemical, to drug, to render it less harmful. This made the toxin or drug water soluble, so it can then be excreted from the body via watery fluids such as bile or urine. Major phase two pathways include glutathione, sulfate, glycine, and glucuronide conjugations. Individual xenobiotics and metabolites usually follow one and two distinct pathways. Again, this makes testing of the various pathways possible by challenging with known substances. The conjugation molecules are acted upon by specific enzymes to catalyse the reaction step. Through conjugation, the iver is able to turn drugs, hormones, and various toxins into excretable substances. For efficient phase two detoxification, the liver cells require sulphur-containing amino acids such as taurine and cysteine (Cabot, 2003).

So, there are several reasons for this. First, the liver of teleosts is the major site of the cytochrome P-450 mediated mixed-function oxidase system. This system inactivates some xenobiotic, while activating others to their toxic forms. Secondly, nutrients derived from gastrointestinal absorption are stored in hepatocytes and released for further catabolism by other tissues (Walton and Cowey, 1982). Third, bile synthesized by hepatocytes aids in the digestion of fatty acids and carries conjugated metabolites of toxicants into the intestine for excretion or enterohepatic recirculation. Finally, the yolk protein, vitellogenin, destined for incorporation into the ovum, is synthesized entirely within the liver (Vaillant et al., 1988).

In the liver of fish, there were many studies about the toxicant of pesticides. For example, Kumar and Sexena (2003) reported to retention of the OP in the liver for days of months after intoxication opposes the usual opinion that such pesticides were quickly degraded in nature. Begum and Vijayaraghavan (1999) reported to the use liver tissue of the fish *Clarias batrachus* (Linn), exposed carbofuran insecticide. The result showed that there were induced biochemical alterations. Fanta et al. (2003) reported that the liver of fish *Corydoras paleatus* contaminated with sublethal levels of OP in water.

Histopathology of fish liver

Pesticides may find their way into the water reservoirs, streams, and river, thus producing an adverse impact on the aquatic biota including fishes. Continuous accumulation of these pesticides may bring about several changes in fish resulting into pathological conditions. It can also alter the normal activity of fish by changing their physiology (John and Prakash, 2003). Environmental studies using fish as sentinel organisms are not new with investigations reported of varying magnitude. In each of these, analysis of histological alterations played a major role. However, seasonal changes should be considered to present errors in histopathological analysis (Hinton, 1993). Diagnosis and prediction of physiological consequences of sublethal contamination can be obtained through histopathology.

Anees (1978) performed the evaluation of histological liver damage on fish *Channa punctatus* (Bloch) that was exposed to sublethal and chronic levels of three OPPs (diazinon, methyl parathion, and dimethoate). The results indicated the damage to the hepatic blood supply and the appearance of dark, granular cytoplasmic inclusions exposure with diazinon. Rodrigues and Fanta (1998) reported sublethal levels of Dimethoate 500 (OPP) to histological change in the liver of *Bracchydanio rerio.* The histological damages were the loss of the typical polygonal cell shape and of detectable cell limits, lateral migration of nuclei, nuclear size and shape, condensation of chromatin and pycuosis, increased cytoplasmic granulation, cloudy swelling, density of granules, and focal necrosis. Fanta et al. (2003) reported sublethal levels of OP methyl parathion exposed on the liver of freshwater teleost *Corydoras pateatus*, which found the liver exhibited cloudy swelling, bile stagnation, focal necrosis, atrophy, and vacuolization.

Barroso et al. (2003) used the presence of histological lesions in liver of catfish (*Ariopsis assimilis*) from the bay of Chetumal, which found a high prevalence of cellular and histological alteration in liver, including hepatic tumors. Histological studied of rainbow trout *Oncorhyncus mykiss*, the snake head *Channa punctatus*, walking catfish *Clarius batrachus*

and tilapia *Saratherodon mossambicus* indicated malathion causes necrosis, hyperplasia, and edema of gills with vacuolation and necrosis of liver (Walsh and Ribelin, 1975; Dubale and Shah, 1979; Shukla et al., 1984). Moreover, Areechon and Plumb (1990) studied effect of a malathion solution on channel catfish *Ictalurus punctatus*. The result, the liver histopathological effect of a sublethal concentration occurred vacuolation and focal necrosis.

DDT poisoing can be associated with effect on the morphologic change in animal liver including hypertrophy of hepatocytes and subcellular organelles such as mitochondria, proliferation of smooth endoplamic reticulum and the formation of inclusion bodies, centrolobular necrosis following exposure to high concentrations, and an increase in the incidence of hepatic tumors (Klaassen, 2001).

Acute and extensive necrosis of liver cell may occur in toxic conditions. Generalized swelling and pyknosis of hepatocyte nuclei, often with cytoplasmic vacuolation, is also commonly found in toxic conditions. Histological extreme cases may show distension of every liver cell by a single large globule of fat and macrophage invasion with ceroid or lipofuscin deposition also occur. The most frequently encountered types of degenerative changes were those of; hydropic degeneration, cloudy swelling, vacuolation, focal necrosis, pyknosis, karyorrhexis, karyolysis, fatty degenerative changes, zonal, massive, and pericentral necrosis, cirrhosis, malignant hepatoma, and laminar or subcapsular necrosis (Roberts, 1978).

Klong 7, Pathum Thani Province

History of the areas encompassing primarily both sides of the southern part of the Chao Phraya River basin, the mission launched by digging up the new main canal on 9th March 1890. Altogether numbering 43 canals in 1894 Klong 7 is one canal of all. The people move in great numbers until large communities of great wealth in rice production

and local trade were formed as the eventual result, being provided with enough surface water all the year round and convenient trade routes (National Science Museum, 2001).

Freshwater fishes of study area: topographically, the Rangsit Great Plain is a wide expanse of lowland that inclining slightly in the north/southwest direction, and also being officially managed in the South Rangsit Irrigation Project. Bounded on both sides by the western Chao Phraya River and the eastern Nakhon Nayok River, between which a network of smaller irrigation canals have been connected with the purposes to irrigate the whole agricultural areas and to protect the Bangkok Metropolitan from annual flooding by acting as a main water-holding area. Its main portions comprise more or less waterlogged fields, and marshes which are suitable habitats for great many forms of aquatic lives for their living, spawning and nursing young in the extremely plentiful stocks of natural food. Rangsit Great Plain is unquestionably regarded as one of the most productive spots of fishery resources in the central plain of Thailand.

Close relationship between fishes and Rangsit people. Fish is the readily available and cheap protein source that can be taken easily along the canals and in the waterlogged fields; the local fishermen utilize many kinds of fishing gears, according to the suitability of the sites and the desired fish species. The Klong 7, in the rainy season the water level in these canals is high, and together with the appearances of high fish diversity and numerous fish individuals.

Gas chromatography (GC)

GC is used most commonly for the separation and quantitation of organic toxicants. This system consists of an injector port, oven, detector, amplifier (electrometer), and supporting electronics. Current modern gas chromatography uses a capillary column to effect separation of complex mixtures of organic molecules and has replaced, to a large extent, the 'packed' column. Instead of coating an inert support, the stationary phase is coated onto the inside of the column. The mobile phase in an inert gas (called the carrier

gas) usually helium or nitrogen that passes through the column. When a sample is injected, the injector port is at a temperature sufficient to vaporize the sample components with respect to the stationary phase; the components separate and are swept through the column by the carrier gas to a detector, which responds to the concentration of each component. The detector might not respond to all components. The electronic signal produced as the component passes through the detector is amplified by the electron meter, and the resulting signal is sent to a recorder, computer, or electronic data-collecting device for quantitation (Hodgson and Levi, 1997). Many researches were reported to use GC such as; this GC procedure is suitable for quantitative determination of the following specific compounds: BHC, lindane (γ-BHC), heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, captan, DDE, DDT, methoxychlor, and endosulfan (Franson, 2005).

In electron capture (ECD) is used to detect halogencontaining compounds, although it will produce a response to any electronegative compound. When a negative DC voltage is applied to a radioactive source (e.g., ^{63}Ni and ^{3}H), low –energy β particles are emitted, producing secondary electrons by ionizing the carrier gas as it passes through the detector. The secondary electron stream flows from the source (cathode) to a collector (anode), where the amount of current generated (Called a standing current) is amplified and recorded. As electronegative compound pass from the column into the detector, electrons are removed or 'captured', and the standing current is reduced. The reduction is related to both the concentration and electronegativity of the compound passing through, and this produces a response that is recorded. The sensitivity of ECD is greater than of any other detectors currently available (Hodgson and Levi, 1997).

CHAPTER III

MATERIALS AND METHODS

Study area and Sample

Study area

The study area is Klong 7, Pathum Thani Province which is located in the lower plain of Pasak and Chao Phraya river basins, north of Bangkok. Rangsit irrigation system, situated east of Pathum Thani province, composes of 14 sub-canals (klong). Each klong is 20 km long and Klong 7 is at the center of the irrigation system. Rangsitprayulasakdi canal, situated along the southern end of each sub-canal, is the main canal that receives water from sub-canals and transfers water to the Chao Phraya River which flows towards Bangkok.

Klong 7, located in Nong Sua district, Prathum Thani province is one of 14 sub-canal used for irrigating Rangsit agricultural areas more than 100 years (mainly products i.e. rice, sugarcane, tapioca, and tropical fruits). This area is undoubtedly one of the most contaminated spot of pesticides in the central plain of Thailand.

Klong7 and other sub-canals receive water from Raphi Phat Yaek Tok canal, north of Prathum Thani province. So, the contaminants from both nearby agricultural areas and from Raphi Phat Yaek Tok canal mix into this canal and flow to Rangsitprayulasakdi canal towards Chao Phraya River at Bangkok.

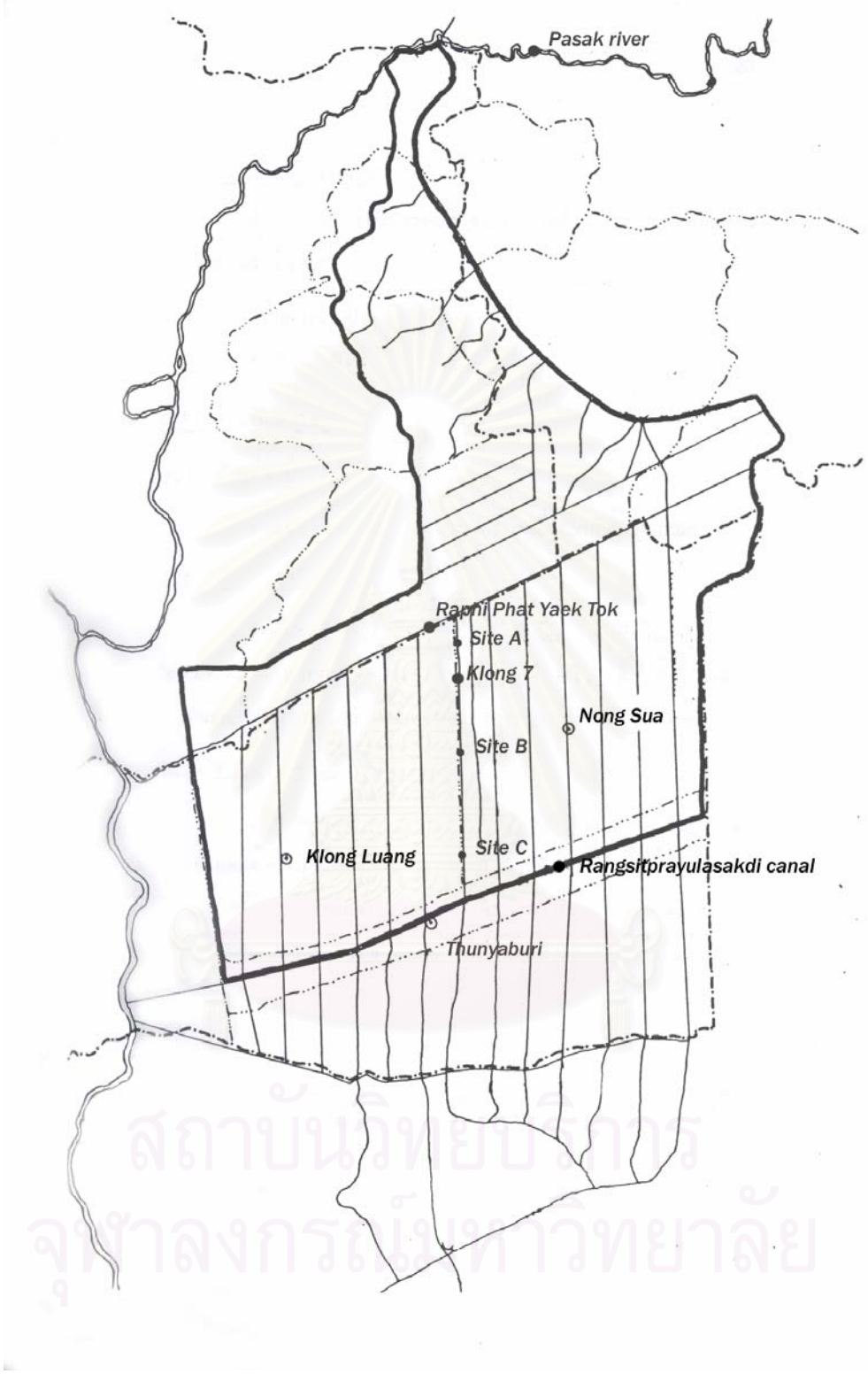


Figure 3-1 Map of Rangsit irrigation system, showing Klong 7 and the position of the sampling sites (Disyawong, 1979).

Collection of samples

Water and fish samples were collected from the Klong 7, Pathum Thani Province. Water samples were collected from March to December 2004 (one time/month). Fish samples (body length 3.3-5.0 cm.) were collected at summer on March and April 2004 (one time/month) and at rainy season on May to August 2004 (one time/month). Three replicates of water samples were collected at each site by 1 liter polyethylene bottles. Then, water samples were measured for pH, DO (dissolved oxygen), and temperature. After that, they were stored in an ice box and transported to the laboratory and stored in 4^{o}C until extraction (Graves, 1989). Fish samples were collected at three sites but were combined into one group. They were collected at the mature stage. The body weight (g) and length (cm) was record before shocked at 0^{o}C. Then, the abdomen of each fish was opened. The liver were removed and measured for weight (g), then it was fixed in 10% neutral buffered formalin.

Figure 3-2 Siamese glassfish *Parambassis siamensis* at mature state.

Fish study (control and a positive control)

Control fish

The Siamese glassfish *Parambassis siamensis* adult state (body length 3.3 - 5.0 cm.) used in this study was obtained from Narongdach Farm, Ratchaburi Province. They were observed in 325 liter glass aquaria for 8 weeks prior to exposure and fed with the brine shrimp once a day.

Holding conditions

Tap water was filtered through carbon-resin filter, aerated and finally used as holding water in this experiment. Water conditions including temperature, dissolved oxygen (DO), and pH were measured routinely throughout the test. Water temperatures in control and treatment aquaria were similar, ranged between 26 and 28°C. Dissolved oxygen ranged between 5.4 and 5.8 mg/L in both groups (control and endosulfan treated groups). The pH value in control water ranged between 7.0 and 7.4 as same as endosulfan treated water.

Pesticide used in the experiment

Endosulfan (E-TO®; TJC Chemical Company, Thailand) has chemical formula (1, 4, 5, 6, 7, 7-hexachloro-8, 9, 10-trinorborn-5-en-2, 3-ylenebismethylene) sulfile 35% w/v EC, which was dissolved directly into holding water to yield the desired concentrations at 0.01, 0.06, 0.5, and 1 ppb. Figure 3-3 shows the commercial endosulfan pesticide used in this experiment.

Figure 3-3 E-TO®, an endosulfan from commercial source.

A positive control

A positive control was designed for comparative study between summer group and rainy season group. Endosulfan sulfate is one of many pesticides found in Klong 7. Endosulfan sulfate is metabolized from endosulfan by fish in 24 hours (Mastsumura, 1976). Siamese glassfish brood stock adult state was obtained from the Narongdach Farm, Ratchaburi Province and was acclimated for 8 weeks in 325 liters glass aquaria. The test was conducted in 14 liters glass jars containing 10 liters of different concentration level of the endosulfan solution. Four jars were filled with the treated solution at 0.01, 0.06, 0.5, and 1.0 μg/L endosulfan. All experimental conditions were duplicated and 5 fish were used for each jar. The experiment was carried out for 8 days. Then, fish were shocked at 0°C. The abdomen of each fish was opened for liver collection. Body weight (g), liver weight (g), and total length (cm) was recorded. All livers were fixed in 10% neutral buffered formalin for 48 hours and proceeded for permanent slide. LC_{50} of the eel *Anguilla anguilla* was shown at 41 μg/liter (ppb) of endosulfan (Gimeno et al, 1995).

Histological analysis

1. The preparation of fish liver for standard paraffin technique was done by cutting the liver into small pieces about 0.5-1 cm^3, and fixed in 10% neutral buffered formalin for 24 hours at room temperature. After that the tissues were dehydrated in the series of ethanol and embedded in paraplast (Figure 3-4). The paraffin blocks were cut at 5 μm thickness by the rotary microtome; the sections were attached with the slides and dry at 40°C on a warm plate. These sections were deparaffined with xylene and hydrated in the series of ethanol before staining by haematoxylin and eosin (H&E) (Figure 3-5) (Gurr, 1963) and then examined under Photomicrograph Axioskop 40, Evolation™ LC.

2. The frozen technique is applied for the histochemistry study. The liver was cut into small pieces, approximate size of 3 mm^3, and then rapidly put it in the foil mold and filled with the frozen medium (tissuetek). The tissue block was cut at 5 μm tickness by the cryostat. The sections were set on tissue block the slides and air dry before staining.

- The Oil red O staining technique was used for the study on the lipid accumulation of the fish liver (Culling, 1963). The staining procedure is shown in Figure 3-6.

- The Periodic Acid/Schiff (PAS) technique was used for identified glycogen content (Gurr, 1963). The staining procedure is shown in Figure 3-7.

Permanent H&E staining slide of all groups were study under light microscope.

Criteria for histological analysis for Oil red O staining are classified by

Density of lipid droplets

Mild	1-25% lipid droplet of total area
Moderately	26-50% lipid droplet of total area
Strongly	51-75% lipid droplet of total area
Extremely	76-100% lipid droplet of total area

Size of lipid droplet classification in very small, small, and large size, respectively and all of them are criterion for histological analysis.

For PAS staining in the liver tissue of summer group, rainy season group, and treated endosulfan group were compared with the liver tissue of the control group.

Data analysis and statistical procedures

Calculations of body weight and liver weight were performed on computer by Microsoft Excel for Windows XP.

Statistical analysis of the data was performed by SPSS program version 11.0. Oneway ANOVA was used to determine significant differences at $p \leq 0.05$ of the relative liver weigh index of all groups. The observed significances were then confirmed with LSD and Duncan test. P values of ≤ 0.05 were considered significant.

Figure 3-4 The tissue embedding procedure (Gurr, 1963)

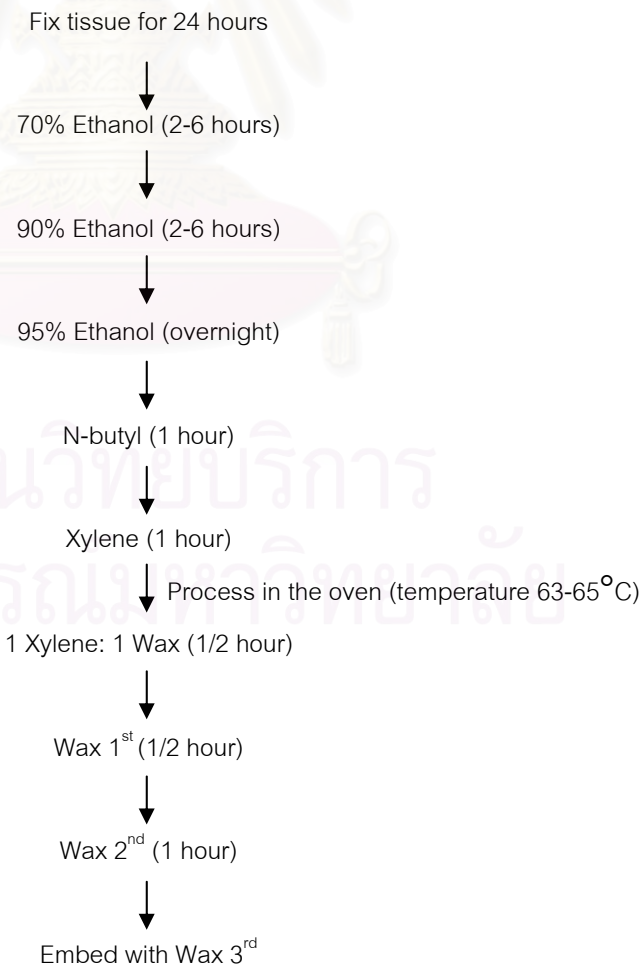


Figure 3-5 The haematoxyline and eosin staining procedure (Gurr, 1963)

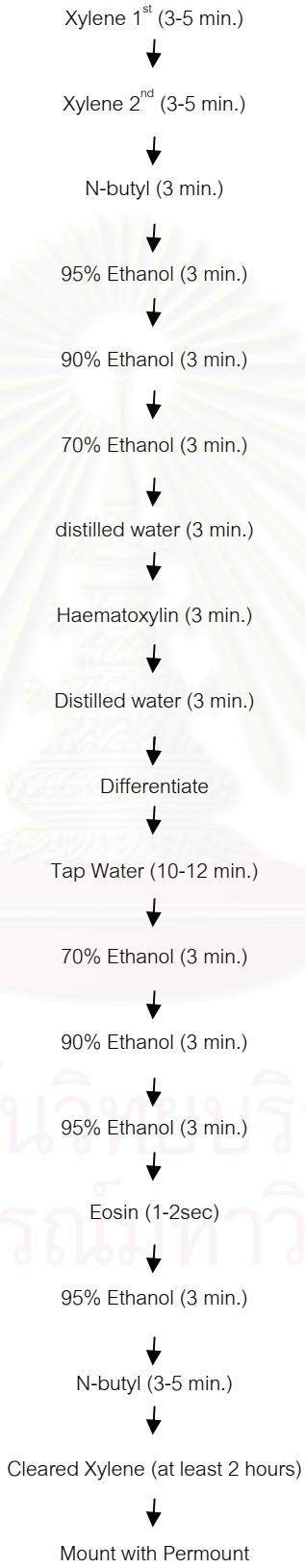


Figure 3-6 The Oil red O staining procedure (Gurr, 1963)

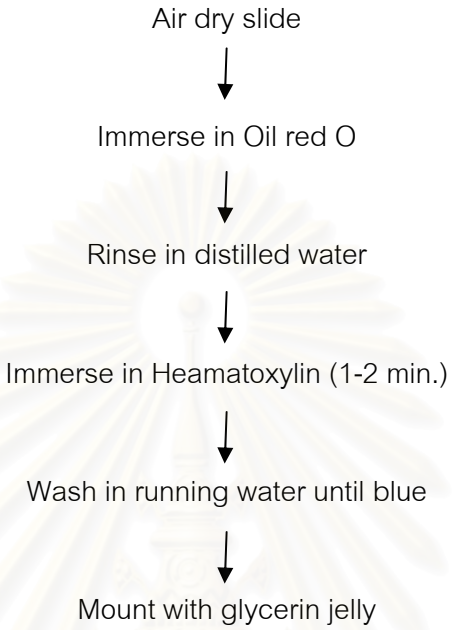
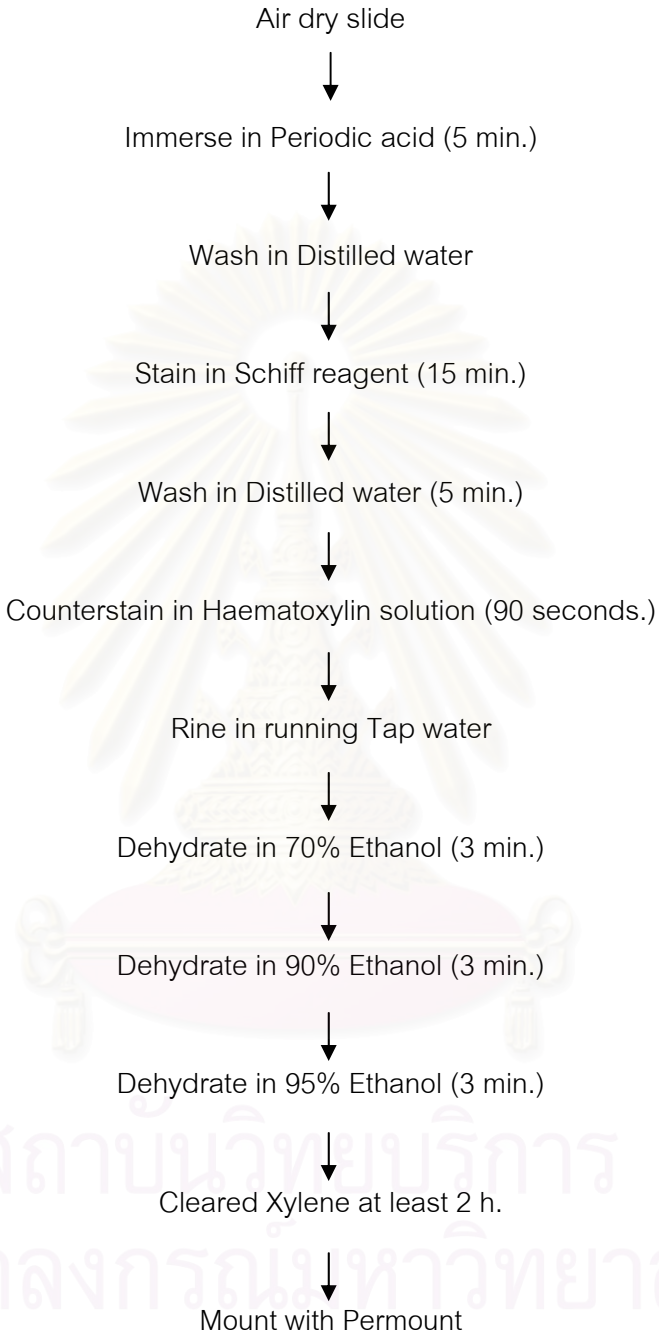


Figure 3-7 The Periodic acid/Schiff (PAS) procedure (Gurr, 1963)



Analysis of the water samples

Gas Chromatography

Gas Chromatographic (GC) method for the determination of certain chlorinated pesticides in wastewater is comparable to USEPA method 508.

Water sample extracts were analyzed using Hewlett Packard HP-6890N gas chromatographs that have autosample injector in 7683 Series and Gas Chromatography micro Electron Capture Detector (GC-μECD).

Condition;

Analytical Column	DB 35MS, capillary column J&W Scientific Company
Temperture Program	100^{o}C (2 min) rate to 12^{o}C/min to 280^{o}C
Injection Mode	Spiltless mode, Split vent at 60 ml/min, Heater 260^{o}C, Pressure 23.87, and Total Flow 64.6 ml/min
Detector	300^{o}C, Micro Electron Capture Detector (μECD)
Gas	carrier gas: He, flow rate 2 ml/min Make up gas: N_2 ,flow rate 60 ml/min
Injector volume	1 μl
Analytical data	Software Chemstation of Agilent Technologies

Calibration and Standardization

The GC system must be calibrated using the external standard technique as follows:

External Standard Calibration Procedures

Stock calibration standards or 17 mixed standards were prepared at 10 μg/L from 200 μg/L of pesticides. The mixed standard was diluted with hexane. The range of the pesticides standards was from 2, 5, 10, 20, and 50 μg/L. Analysis of pesticide standards for calibration curve was conducted by GC-μECD. The order and peaks of Organochlorine Pesticides (OCPs) were shown in the chromatogram. The area of each peak was measured and plotted against their concentrations obtain the calibration curve for each compound.

Procedure

Extraction (Modified from USEPA Method 608)

1. Mark 800 ml the water meniscus on the side of the sample bottle for later determination of sample volume.
2. Add 60-mL hexane to the sample bottle, seal, and shake 30 s to rinse the inner walls. Transfer 100 mL the (1:1) dichloromethane: hexane solvent to the separatory funnel and extract the sample by vigorously shaking the funnel for 3 minutes with periodic venting layer to separate from the water phase for a minimum of 10 min. Collect the solvent extract in a 500 mL TurboVap® tube by transferring sodium sulphate anhydrous.
3. Transfer 50 mL the dichloromethane: hexane solvent to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the TurboVap® tube by transferring sodium sulphate anhydrous.
4. Repeat in step 3.

Extract Concentration

The extracted sample was transferred into the TurboVap® tube and concentrated into the final volume 2 mL at constant temperature 40°C. Afterward, the concentrated sample was kept in TFE-fluorocarbon screw-cap vial, then it was stored in the refrigerator at -5°C before analysis by GC-μECD.

Identification, Data analysis, and Calculation

One microlitre was injected into GC. If an extracted sample contains organochlorine pesticides (OCPs), it can be analyzed the quality and quantity of each OCPs by the comparison of the retention time and peak area with OCPs calibration curve standard. The determination of OCPs quantity can be calculated as described by linear regression equation.

Quality control

The major parameters that should be known and kept in control in order to generate quality analytical data, the precisions of the measurement must be defined and checked to be sure it is remaining stable. This is done by measuring replicate samples. Possible contaminated samples also must be controlled. The running of blank ensures that each step is no contamination or interference. A mix standard spike or standard reference material is used to ensure that the analyze is being properly extracted and accurately quantified. Each of these areas of control must be check more frequently. The minimum requirements of this experiment consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blank as test of continued performance and analysis of spiked samples to assess accuracy

Blank

Distilled water were extracted with extraction procedure, then extracted distill water was injected into GC for analysis OCPs contamination.

The percentage recovery

1. Distilled water 800 ml was spiked with mixed organochlorine pesticides standard, so that the final conc. were 5 μg/L (7 replicateds).

2. Spiked water was extracted with extraction procedure.

3. Then, all the final concentration of spiked water were analyzed by GC-μECD and calculated the % recovery follow the equation below;

$$\%\text{recovery} = \frac{\text{Analyzed concentration}}{\text{spiked concentration}} \times 100$$

The Field replications

Three repicated water samples were collected from each site (three sites) of Klong 7 Pathum Thani Province. The amount of OCPs concentrations were determined and reported in each month.

The study of Limit of Detection (LOD) and Limit of Quantitation (LOQ) of GC-μECD

The Limit of Detection (LOD) and the Limit of Quantitation (LOQ) were determined by injection of mixed standard solution, were 10, 1.0, 0.1, 0.01, 0.001 μg/L. The LOD and LOQ were calculated follows the equation below;

LOD = 3 S/N (signal/noise)

LOQ = 10 S/N

CHAPTER IV

RESULTS AND DISCUSSION

Gross anatomy of Siamese glassfish *Parambassis siamensis* liver

The liver of the control group was normal in structure. It had a single lobe which connects to the digestive tract and its color varies from pale yellow to yellow. The gross anatomy of the liver of Klong 7 and endosulfan treated fish were yellow to yellowish brown. The result supported the experiment of Grizzle and Rogers in 1976. Schlenk and Benson (2001) reported that most gross inspection of livers of fish were in the form of a single lobe. In contrast, the liver structure of the Common Silver Barb is not clearly separated into lobe and does not contact with the digestive tract (Hinton, 1993).

Relative liver weight index (%R)

The percent relative liver weight of control group, Klong 7 summer group, Klong 7 rainy season group, 0.01 μg/L endosulfan treated group, 0.06 μg/L endosulfan treated group, 0.5 μg/L endosulfan treated group, and 1 μg/L endosulfan treated group were 1.334 $\pm$ 0.162, 2.319 $\pm$ 0.094, 1.321 $\pm$ 0.164, 1.326 $\pm$ 0.145, 1.235 $\pm$ 0.127, 1.564 $\pm$ 0.221, and 1.733 $\pm$ 0.236, respectively. From the statistical analysis using Oneway ANOVA, the percent relative liver weight of Klong 7 group in summer showed significant different from control group ($p \leq 0.05$) (Table 4-1).

The importance of fish liver for organismic homeostasis and environmental acclimation is already evident with the relationship between weight of liver and that of body weigh is relative liver weight index (%R). The fish liver of Klong 7 at summer group was significantly higher than the control group ($p \leq 0.05$). Normally, the relative liver weight index of common fish should be between 1-2% (Roberts, 1978). In this study, the ratio of summer group is over the standard ratio. Schlenk and Benson (2001) reported that chronic stress by toxicant affected to liver cells may undergo an adaptive hyperplasia, resulting in

an increase of the relative liver weight index. So that, the fish liver in summer may be affect by toxicant in water of *Kong 7*, Pathum Thani Province.

Table 4-1 Body length, body weight, liver weight, and %relative liver weight (%R) of control, Klong 7 summer, Klong 7 rainy season, and endosulfan treated group (Mean ± SE.).

Group	Body length (cm) (Mean ± SE)	Body weight (g) (Mean ± SE)	Liver weight (g) (Mean ± SE)	%R (Mean ± SE)
control (n=30)	3.80 ± 0.09	1.61 ± 0.08	0.0215 ± 0.0029	1.334 ± 0.162
summer (n=60)	4.34 ± 0.05	2.80 ± 0.09	0.0640 ± 0.0027	2.319 ± 0.094 *
rainy season (n=30)	3.96 ± 0.07	1.83 ± 0.09	0.0220 ± 0.0022	1.321 ± 0.164
endosulfan 0.01 μg/L (n=10)	3.35 ± 0.07	1.15 ± 0.07	0.0160 ± 0.0027	1.326 ± 0.145
endosulfan 0.06 μg/L (n=10)	3.40 ±0.09	1.19 ± 0.08	0.0150 ± 0.0022	1.235 ± 0.127
endosulfan 0.5 μg/L (n=10)	3.47 ± 0.07	1.26 ± 0.07	0.0210 ± 0.0041	1.564 ± 0.221
endosulfan 1 μg/L (n=10)	3.68 ± 0.13	1.56 ± 0.12	0.0280 ± 0.0047	1.733 ± 0.236

* Indicate significant differences from all groups ($p \leq 0.05$).

Histological alteration

In normal or control Siamese glassfish liver, polygonal hepatocytes are arranged in longitudinal orientation tube and proceeding from one sinusoidal profile, across the hepatocytes, to the next sinusoidal profile as a mesh-like network form and anastomosing cords. As in other fish, there is no lobulation (Plate 1A – F), a double row of hepatocytes have obvious and rounded nucleus. Each tube is surrounded by a number of blood capillariy or sinusoid, which derives from the portal vein and interpenetrates the entire mass of the liver. Most of the hepatocytes nuclei are located in the central of cell or located basally in well fed liver. The appearance of hepatocyte varies between specimens by the

degree of vacuolation. In well fed fish, liver tissue shows the space of vacuole, which its glycogen and fat are removed by routine tissue preparation.

Klong 7 summer group, liver showed the sign of cellular injury, most of liver lesions were focal necrosis (Plate 2B), few diffuse necrosis (Plate 2A), and infiltration of macrophage around the blood vessel (Plate 2C). Many cases displayed sinusoid dilated (Plate 2D) and blood congestion (Plate 3B), deposition fat droplets (Plate 2D, 2E, and 2F) and accumulation of hyaline granule (Plate 3A). The groups of death cell were seen in some case (Plate 4F). Many cases established detachment of both capsular epithelial lining (Plate 3D) and endothelial of blood vessel (Plate 3E). In some case they were pyknotic cell (Plate 3F), karyolitic nucleus (Plate 3C), and perinuclear chromatin clumping nucleus (Plate 3C and 4A). A few case, fragment of erythrocyte in blood vessel (Plate 4C and 4B), less of erythrocyte in sinusoid (Plate 3E), and mild infiltration of white blood cells into hepatic tissue were seen (Plate 4E). Damaged liver showed disarrangement of hepatic plate (Plate 3D). Pancreatic shrinkage was found in some case (Plate 2A).

Klong 7 rainy season group liver showed cellular injury which was focal necrosis (Plate 5A) and diffuse necrosis (Plate 6A and 6F). Most cases displayed the infiltration of macrophage around the sinusoids (Plate 5B), and a few of erythrocytes in sinusoid (Plate 5C), sinusoid dilated and blood congestion (Plate 5D). Generally, accumulation of fat droplets (Plate 5E) and hyaline granule in cytoplasm (Plate 5B) were noticed. In addition, the group of death cell was seen in some cases (Plate 5F). The detachment of capsular epithelial lining (Plate 6A) and endothelial lining of blood vessel (Plate 6B) were found in many cases. Pyknotic cells with karyolysis or perinuclear chromatin clumping nucleus were noticed in some case (Plate 6C). A few fragments of erythrocyte in blood vessel (Plate 6B) and moderately infiltration of white blood cells in hepatic tissue were observed. Eosinophilic area was evidence in some case (Plate 5D). Hepatic plate disarrangement and eosinophilic area were also seen (Plate 5F, 6 F, and 5C).

Liver of 0.01 μg/L endosulfan treated group showed disarrangement of hepatic plate (Plate 7A, 7B, and 7C), eosinophilic cytoplasm, and sinusoid dilated and blood congestion (Plate 7A). Accumulation of fat droplets and hyaline granule were seen in some case cytoplasm (Plate 7A). Detachment of endothelial lining of central vein (Plate 7B), pyknotic cell, karyolysis nucleus, and perinuclear chromatin clumping (Plate 7D) were seen in few case. A few livers showed morphological change of erythrocyte (Plate 7F). Focal and diffuse necrosis and macrophage invasion were noted. Pancrease distortion was seen in some fish (Plate 7A).

Liver of 0.06 μg/L endosulfan treated group showed moderately disarrangement of hepatic plate (Plate 8F). Eosinophilic cytoplasm, sinusoid dilated and blood congestion, accumulation of fat droplets, and mild accumulation of hyaline granule in cytoplasm were deserved (Plate 8B, 8C, and 8D). Detachment of epithelial lining capsule (Plate 8C) and detachment of endothelial lining, pyknotic nucleus (Plate 8F), karyolysis nucleus (Plate 8D), and perinuclear chromatin clumping were noticed (Plate 8A, 8C, 8D, and 8F). In some case, they were fragmentation erythrocytes in blood vessel (Plate 8B) and focal necrosis (Plate 8A and 8B). Macrophages were seen in some liver.

Liver of 0.5 μg/L endosulfan treated group showed cellular lesion that consist of moderately disarrangement of hepatic plate (Plate 9B, 9C, and 9D). Some liver displayed eosinophilic cytoplasm (Plate 9B), sinusoid dilated and blood congestion, slightly accumulation of fat droplets (Plate 9E), and moderately accumulation of hyaline granule in cytoplasm (Plate 9D). Detachment of epithelium lining capsule and blood vessel were noticed (Plate 9A). In some areas these were pyknotic nucleus, karyolysis perinuclear chromatin clumping (Plate 9D-E). Focal necrosis, diffuse necrosis, polymorphic erythrocytes and macrophage were observed (Plate 9C, 9D, 9E, and 9F).

Liver 1 μg/L endosulfan treated group, histological change composed of disarrangement of hepatic plate, eosinophilic cytoplasm, sinusoid dilated, blood congestion, accumulation of fat droplets, and accumulation of hyaline granule in cytoplasm (Plate 10B-

D). Mild case found detachment of epithelium lining of capsule and detachment of endothelial lining of blood vessel (Plate 10D). Pyknotic nucleus (Plate 10F), karyolysis (Plate 10B), and perinuclear chromatin clumping were seen in most area (Plate 10B and 10F). They also found macrophage and polymorphic red blood cell.

The hepatic cells injury of Siamese glassfish in both summer and rainy season group exhibited the same result and was similar to the effect of malathion, which was also found a decrease in the number of red blood cell in sinusoid of Indian catfish *Heteropneustes fossilis* (Awasthi et al., 2003). Their study revealed that malathion caused an decrease in total erythrocyte count. This study shown liver lesion as same as the pyknotic cell, karyolysis nucleus, and perinuclear chromatin clumping nucleus dimecron (organophosphate insecticide) exposed to *Gambusia affinis*, found the focal necrosis in hepatocytes demonstrated that liver affected to various agents (Schlenk and Benson, 2001). Fanta et al. (2003) reported the effect of methyl parathion on focal necrosis, atrophy, and vacuolization of hepatocyte. Generalized focal necrosis, pyknotic cell, karyolysis nucleus, and zonal necrosis are also commonly found in toxic conditions (Roberts, 1978). The diffuse necrosis found at liver tissue, is commonly observed in CCl_4 acute toxicity studies (Gingerich et al., 1977). Moreover in this study, blood congestion was similar to the effect of sublethal toxicity of $CdCl_2$ (Klaassen and Amdur, 1980). The inflammatory reaction in the liver was indicated by rupture of endothelium of sinusoid, central vein, and capsule of liver (Schlenk and Benson, 2001). This study also founded the infiltration of macrophage and white blood cell. Schlenk and Benson (2001) reported that macrophage immigration and formation of aggregates was seen after toxicant exposure such as, atrazine exposure was associated with immigration of granulocytes. Generally, hyalin droplet deposition, dilation of sinusoid and lipid accumulation, and all cases of damage was similar to Gimeno et al. (1994) who study liver of the eel *Anguila anguila* that was exposed to endosulfan. However, the pancrease shrinkage was also founded as same as the work of Schlenk and Benson (2001) who reported the effect of folidol on Nile tilapia.

Endosulfan is a persistent pesticide and highly hazardous pesticide classify by U.S. Environmental Protection Agency (US EPA). Several chronic effects have been noted for animals exposed to endosulfan, which is most likely to affect liver (Cornell University, 2005). Endosulfan is a persistent insecticide, stable in soil and to the ultraviolet action of sunlight and the residues of endosulfan or its metabolites have been found and associated with the death of fish in aquatic continental system (Gimeno, 1995). Histological alteration detected in liver tissue of Siamese glassfish was the rupture of epithelium of central vein and capsule rupture, sinusoid dilated, blood congestion, accumulation of fat droplets and hyaline granule in cytoplasm, pyknotic cell, karyolysis nucleus, perinuclear chromatin clumping, morphological change of the erythrocytes, focal and diffuse necrosis, and infiltration of macrophage in hepatic tissue, that were the same result of Sastry and Siddiqui (1982) who studied the effect of endosufan on the snake head fish *Channaa punctatus* found the decrease of lipid droplets and glycogen granule in hapatocytes. Nisha and staff (2003) reported the toxic effect of endosulfan 10 μg/L on liver rat, which showed the toxic interference with the biochemistry and histology of rat liver. Similarly, histological alterations have also been characterized in fish *Gymnocorymbus ternetzi*, *Channa gachua*, *Channa striatus*, and mosquito fish *Gambusia affinis* that exposed to endosulfan (Otludil et al., 2004).

Histochemical study

In normal or control Siamese glassfish liver;

The Oil red O staining liver, the lipid droplets accumulation were small size and moderately density of droplets in hepatocytes (Plate 13A).

The PAS staining liver showed the positive staining of glycogen in hepatocyte of normal control fish (Plate 14A).

Klong 7 summer group;

The Oil red O staining hepatocytes showed that the lipid droplets accumulation of summer group liver was large size and more densely than the control liver (Plate13B).

The PAS staining showed that the glycogen accumulation of summer group liver was more than the control liver (Plate 14B).

Klong 7 rainy season group;

The Oil red O staining liver showed that the lipid droplets accumulation of rainy season group liver were mixed with small and large size and were more densely distribution than the control liver (Plate 13C).

The PAS staining liver showed that the glycogen accumulation of rainy season group liver was more than the control group (Plate 14C).

Liver of 0.01 μg/L endosulfan treated group;

The Oil red O staining section showed the lipid accumulation in 0.01 μg/L endosulfan treated group liver. Both of endosulfan treated liver and control group liver showed the small size droplets and moderately distribution of lipid droplets (Plate 13D).

The PAS staining showed that the glycogen accumulation of 0.01 μg/L endosulfan treated livers as same as the control group (Plate 14D).

Liver of 0.06 μg/L endosulfan treated group;

The Oil red O staining showed that the lipid accumulation in 0.06 μg/L endosulfan treated group liver was the small size and moderately density of lipid droplets as same as the 0.01 μg/L endosulfan treated group liver and the control group liver (Plate 13E).

The PAS staining showed that the glycogen accumulation of 0.06 μg/L endosulfan treated group were lesser than the control group (Plate 14E).

Liver of 0.5 μg/L endosulfan treated group;

The Oil red O staining showed that the lipid accumulation in 0.5 μg/L endosulfan treated group was very small size and lesser densities of lipid droplets than the 0.06 μg/L endosulfan treated group and the control liver (Plate 13F).

The PAS staining showed that the glycogen accumulation of 0.5 μg/L endosulfan treated group were lesser than the 0.06 μg/L endosulfan treated group and the control group (Plate 14F).

Liver 1 μg/L endosulfan treated group;

The Oil red O staining showed that the lipid accumulation in 1 μg/L endosulfan treated group was the very small size and the lesser density of lipid droplets than the 0.5 μg/L endosulfan treated group and the control liver (Plate 13G).

The PAS staining showed that the glycogen accumulation of 1 μg/L endosulfan treated group were lesser than the 0.5 μg/L endosulfan treated group liver and the control group (Plate 14G).

Histochemical parameters are very sensitive to sublethal concentrations of many stress agents. So this study chose the amount of glycogen and lipid droplet accumulation as the histochemical parameter. The result of histochemical study in fish liver at Klong 7 both summer and rainy season showed the increasing glycogen accumulation in liver compare to control liver. Increasing of glycogen referred to the abnormal of hepatic function (Khan et al., 2003). Diazol toxicity was studied in female albino rat *Rattus norvegicus* by administering a dose of 6 mg/Kg body wt and shown a significant decline in hepatic glycogen which indicated liver dysfunction which has been considered to be due to the disturbed glycogenesis. Lipid accumulation in liver tissue in this study was increased in both summer and rainy season liver when compare to control group. This result was similar to the effect of monocrotophos (OPP) on *Boleophthalmus dussumieri* there was the increasing in lipid levels. Arellno and staff (2001) studied the effect of the TCDD demonstrated on the sea bream *Sparus aurata* and reported the increasing of lipid droplets and glycogen granules in hepatocytes.

Lipid accumulation in fish liver after exposed to endosulfan showed depleted lipid droplets accumulation in liver of all concentration when compare to control group liver. Sublethal effect of monocrotophos on *Boleophamus dussumieri* was founded alteration of

lipid levels (Patil et al., 1990). Glycogen deposited in liver after treated with endosulfan all concentrations displayed a decrease of glycogen in hepatocytes when compare to control liver. This result was as same as endosulfan exposed eel *Anguilla anguilla* which showed the decrease of glycogen level (Gimeno et al., 1994). Similarly, glycogen stores are decreased after exposure to endosulfan on carp (Schlenk and Benson, 2001).

The liver is the first organ which received chemicals that are absorbed in the gut. The high concentration of xenobiotic-metabolizing enzymes in the liver was the cytochrome P450-dependent monoxygenase system. Although most biotransformations are detoxication reaction but many oxidative reactions produce reactive metabolites that can induce lesions within the liver. The type of injuries to the liver depend on the type of exposure, whether acute of chronic (Hodgson, 1997). This study found that treated endosulfan group have a few damage of liver than summer and rainy season group at Klong 7. Lipid droplet accumulation was founded in summer, rainy season, and treated endosulfan group, which referred to the abnormal accumulation of fat in hepatocytes. Although many toxicants may cause lipid accumulation in the liver, the mechanisms may be different. Basically, lipid accumulation is related to disturbances in either the synthesis or the secretion of lipoproteins. Heamatological lesions such as erythrocyte degeneration was founded in summer, rainy season, and all treated endosulfan group, which may related to microsomal cytochrome P450 (CYPIA) levels. However, P450 content correlated with some chlorinated compounds (Zapata et al., 2000). Entry of this organochlorine pesticide into the fish and its subsequent accumulation in such metabolically sensitive tissues as liver are expected to produce disturbances in the tissue metabolism (Gimeno et al., 1994). This study founded that the accumulation glycogen level was changed in summer, rainy season, and all endosulfan treated group. So, the metabolism system of liver was altered. Cell necrosis is a degenerative process leading to cell death. Necrosis was usually found in an acute injury and may be a focal necrosis or it may involve in entire lobe as diffuse necrosis. In this study necrosis in all groups (summer, rainy season, and treated endosulfan group) were found. So that cell death occurs, along with rupture of the plasma membrane, accumulation of triglycerides, swelling of mitochondria with disruption of cristae, and

dissolution of organells and nucleus (Hodgson, 1997). Chronic in flammation can be considered to be inflammation of prolonged duration (weeks to months to years) in which active inflammation, and tissue injury and the chronic inflammation which is distinguished by vascular changes, a largely macrophage and white blood cell infiltrate, and tissue destruction (Kumar et al., 2004). This study indicated that summer and rainy season group may demonstrate chronic inflammation while the endosulfan treated group have a few infiltrated of macrophages.

Table 4-2 Number of histological alterations observed in Siamese glassfish *Parambassis siamensis* liver from summer group, rainy season group, and endosulfan treated group.

	control group	summer group	rainy season group	endosulfan treated group			
				0.01 μg/L	0.06 μg/L	0.50 μg/L	1.00 μg/L
Total of fish	20	30	30	10	10	10	10
Hepatocyte							
Accumulation of hyaline granule							
no	20	8	12	6	4	3	4
mild	0	18	10	3	6	4	6
moderate	0	4	5	1	0	3	0
strong	0	0	3	0	0	0	0
Accumulation of lipid							
no	20	1	5	8	8	7	6
mild	0	13	10	2	2	3	4
moderate	0	14	9	0	0	0	0
strong	0	2	6	0	0	0	0
Hepatic necrosis							
Non necrosis	20	4	5	5	3	1	1
Foci necrosis	0	12	8	4	5	4	5
Diffuse necrosis	0	10	10	1	0	4	2
Foci and diffuse necrosis	0	4	7	0	2	1	2
Sinusoid dilution							
no	20	3	8	7	7	5	4
mild	0	16	13	3	3	4	5
moderate	0	10	7	0	0	1	1
strong	0	1	2	0	0	0	0
Sinusoid and blood vessel congestion							
no	20	13	18	6	8	4	4
found	0	17	12	4	2	6	6
Detachment of endothelial lining of blood vessel							
no	20	13	17	7	5	4	4
mild	0	12	9	2	5	5	5
moderate	0	6	4	1	0	1	0
strong	0	1	0	0	0	0	0
Macrophage in liver tissue							
no	20	8	16	9	9	7	7
mild	0	20	13	1	1	3	3
moderate	0	1	1	0	0	0	0
strong	0	1	0	0	0	0	0

Table 4-2 Number of histological alterations observed in Siamese glassfish *Parambassis siamensis* liver from summer group, rainy season group, and endosulfan treated group (Cont.).

	control group	summer group	rainy season group	endosulfan treated group			
				0.01 μg/L	0.06 μg/L	0.50 μg/L	1.00 μg/L
Total of fish	20	30	30	10	10	10	10
Detachment of epithelial lining of liver capsule							
no	20	16	18	7	8	4	4
mild	0	7	10	3	2	6	6
moderate	0	4	2	0	0	0	0
strong	0	3	0	0	0	0	0
Pyknotic cell							
no	20	8	18	8	5	7	3
mild	0	19	12	2	5	3	4
moderate	0	2	0	0	0	0	3
strong	0	1	0	0	0	0	0
Karyolysis nucleus							
no	20	1	2	4	2	2	2
mild	0	10	13	6	7	8	5
moderate	0	16	12	0	0	0	3
strong	0	3	3	0	0	0	0

Mild 1%-33% of total area

Moderately 34%-66% of total area

Strong 67%-100% of total area

Plate 1

Normal fish liver

(H&E staining)

Figure A Photomicrograph of the control liver shows the normal structure which composed of hepatic plate with a double row of hepatocytes (H), each tube is surrounded by sinusoid (S). Most of the hepatocytes nuclei are basally located near sinusoid. Pancreas (P) insert in liver parenchyma.

Figure B Photomicrograph of the control liver shows normal hepatocyte (H), hepatic plate, central vein (CV), and sinusoid (S).

Figure C Photomicrograph of the control liver shows the normal blood vessel (BV) and hepatocyte (H).

Figure D Photomicrograph of the control liver shows simple squamous epithelial lining of the capsule (→) and hepatocyte (H).

Figure E Photomicrograph of the control liver shows the simple squamous epithelial lining of the central vein (→), the normal hapatocyte (H), sinusoid (S), and central vein (CV).

Figure F Photomicrograph of the control liver shows the normal sinusoid (S), with a row of erythrocytes.

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein, BV: Blood vessel, and P: Pancreas

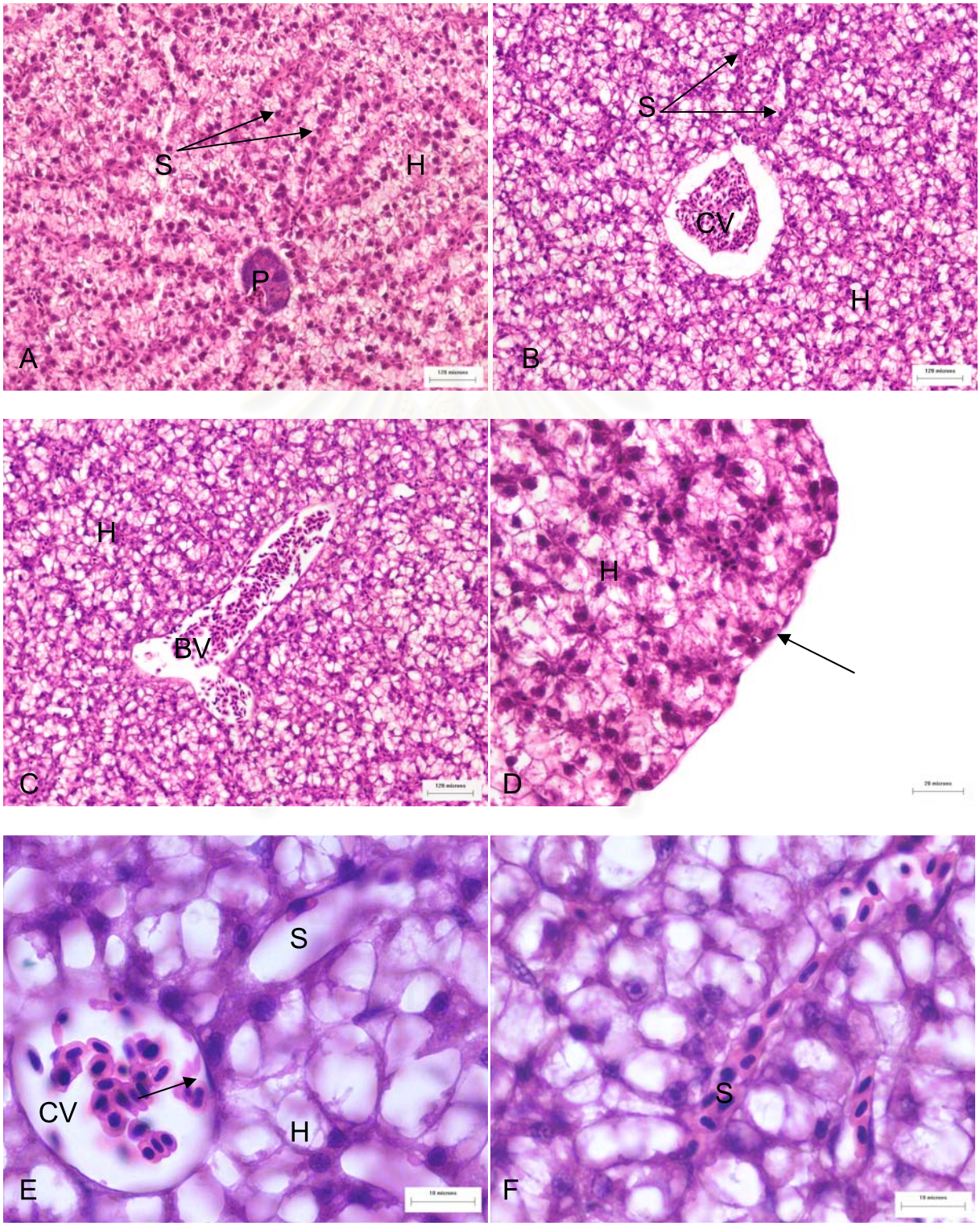

S
H
P
A
S
CV
H
B
H
BV
C
H
D
S
CV
H
E
S
F

Plate 2

Liver of Klong 7 in summer fish

(H&E staining)

Figure A Photomicrograph of liver of summer group shows diffuse necrosis (*) and hyaline granule accumulation (>). Pancreatic shrinkage is showing (P).

Figure B Photomicrograph of liver of summer group shows focal necrosis (*) and accumulation of lipid droplet (>).

Figure C Photomicrograph of liver of summer group shows blood vessel (BV) and macrophage infiltration around the blood vessel (→).

Figure D Photomicrograph of liver of summer group shows sinusoid dilated (→) and accumulation of lipid droplet in cytoplasm (*).

Figure E Photomicrograph of liver of summer group shows lipid droplets aggregation (→).

Figure F High magnification of photomicrograph of liver of summer group shows lipid droplet aggregation (→).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein, and BV: Blood vessel

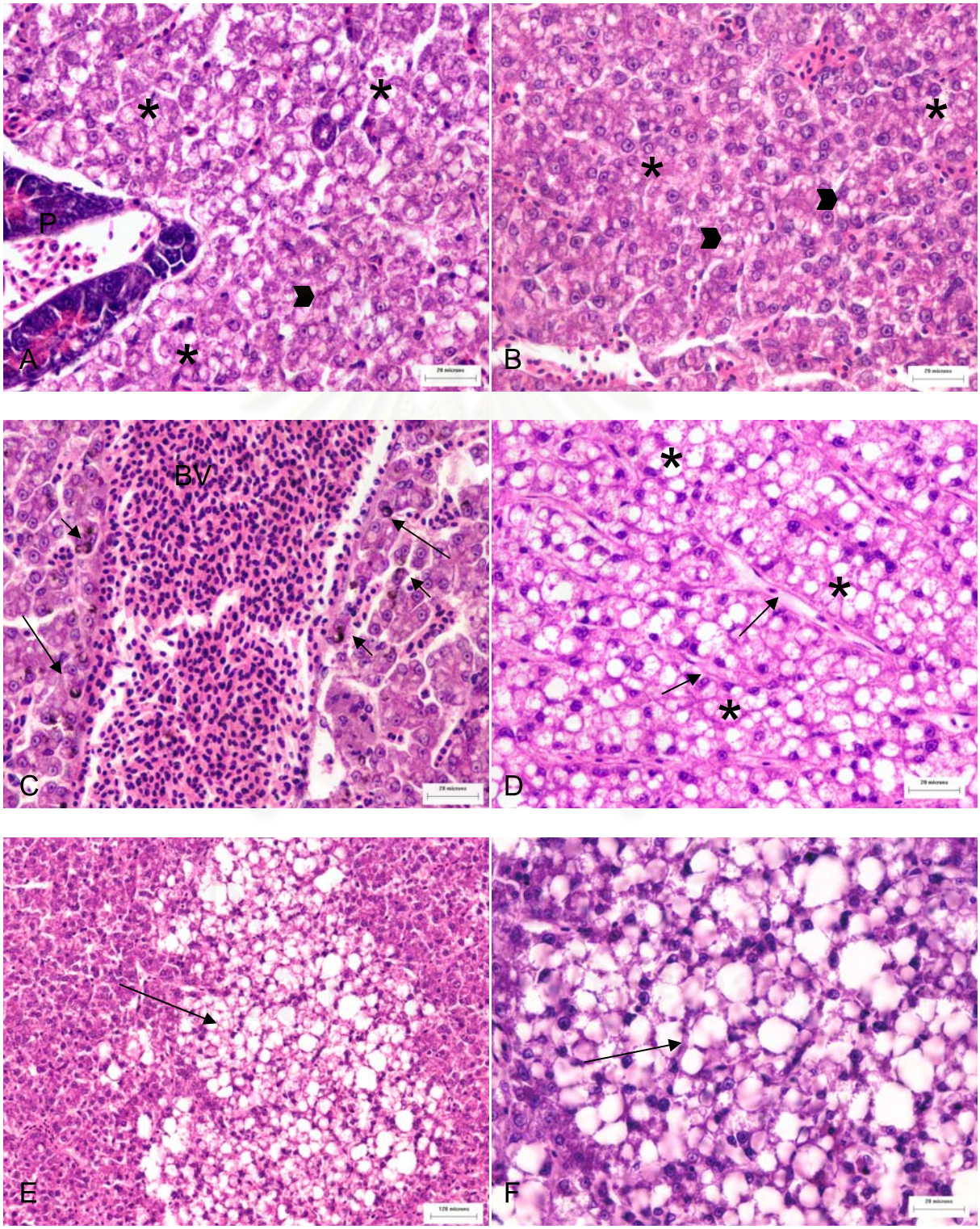

P
A
B
BV
C
D
E
F

Plate 3

Liver of Klong 7 in summer fish

(H&E staining)

Figure A Photomicrograph of liver of summer group shows hyaline granule (>), focal necrosis (*), and blood vessel (BV).

Figure B Photomicrograph of liver of summer group shows blood congestion in sinusoid (→) and karyolysis (>).

Figure C Photomicrograph of liver of summer group shows karyolysis (→) and perinuclear chromatin clumping nucleus (>).

Figure D Photomicrograph of liver of summer group shows the detachment of epithelial lining of capsule (→) and disarrangement of hepatic plate (*).

Figure E Photomicrograph of liver of summer group shows the detachment of endothelial lining (→) of blood vessel (BV) and a few of erythrocyte in sinusoid (>).

Figure F Photomicrograph of liver of summer group shows pyknotic nucleus (>) and eosinophilic cytoplasm of hepatocytes (H).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein,
BV: Blood vessel, BD: bile duct, and P: Pancreas

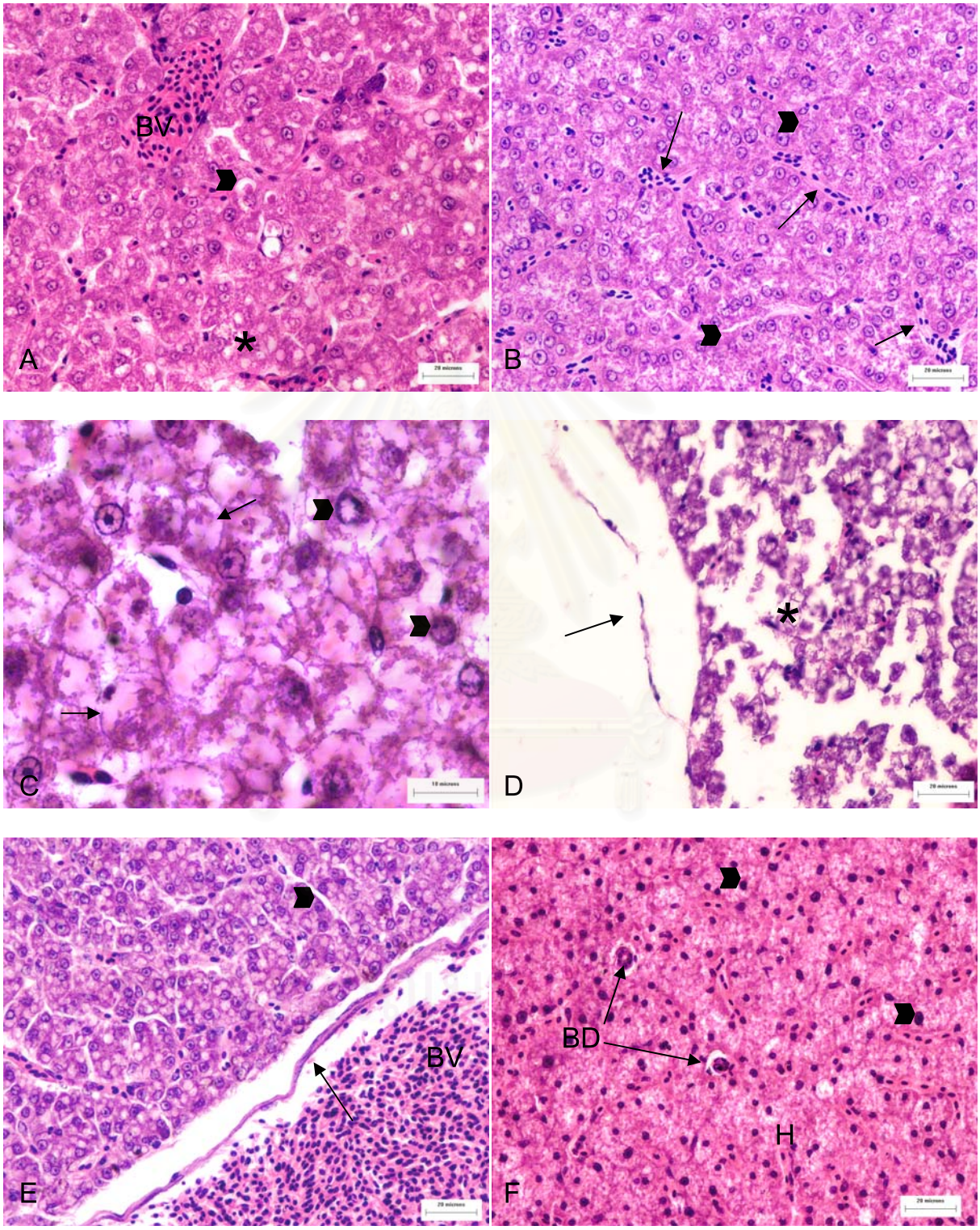

BV
A
B
C
D
BV
E
BD
H
F

Plate 4

Liver of Klong 7 in summer fish

(H&E staining)

Figure A Photomicrograph of liver of summer group shows perinuclear chromatin clumping nucleus of hepatocyte (→) and infiltration of macrophage (>).

Figure B Photomicrograph of liver of summer group shows groups of necrotic erythrocytes in central vein (*).

Figure C Photomicrograph of liver of summer group shows fragment of necrotic erythrocytes in blood vessel (F).

Figure D Photomicrograph of liver of summer group shows perinuclear chromatin clumping nucleus (→) and karyolysis (*).

Figure E Photomicrograph of liver of summer group shows the infiltration of white blood cells in hepatic parenchyma (→).

Figure F Photomicrograph of liver of summer group shows groups of necrotic cell in fibrous capsule (*).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein,
BV: Blood vessel, and P: Pancreas

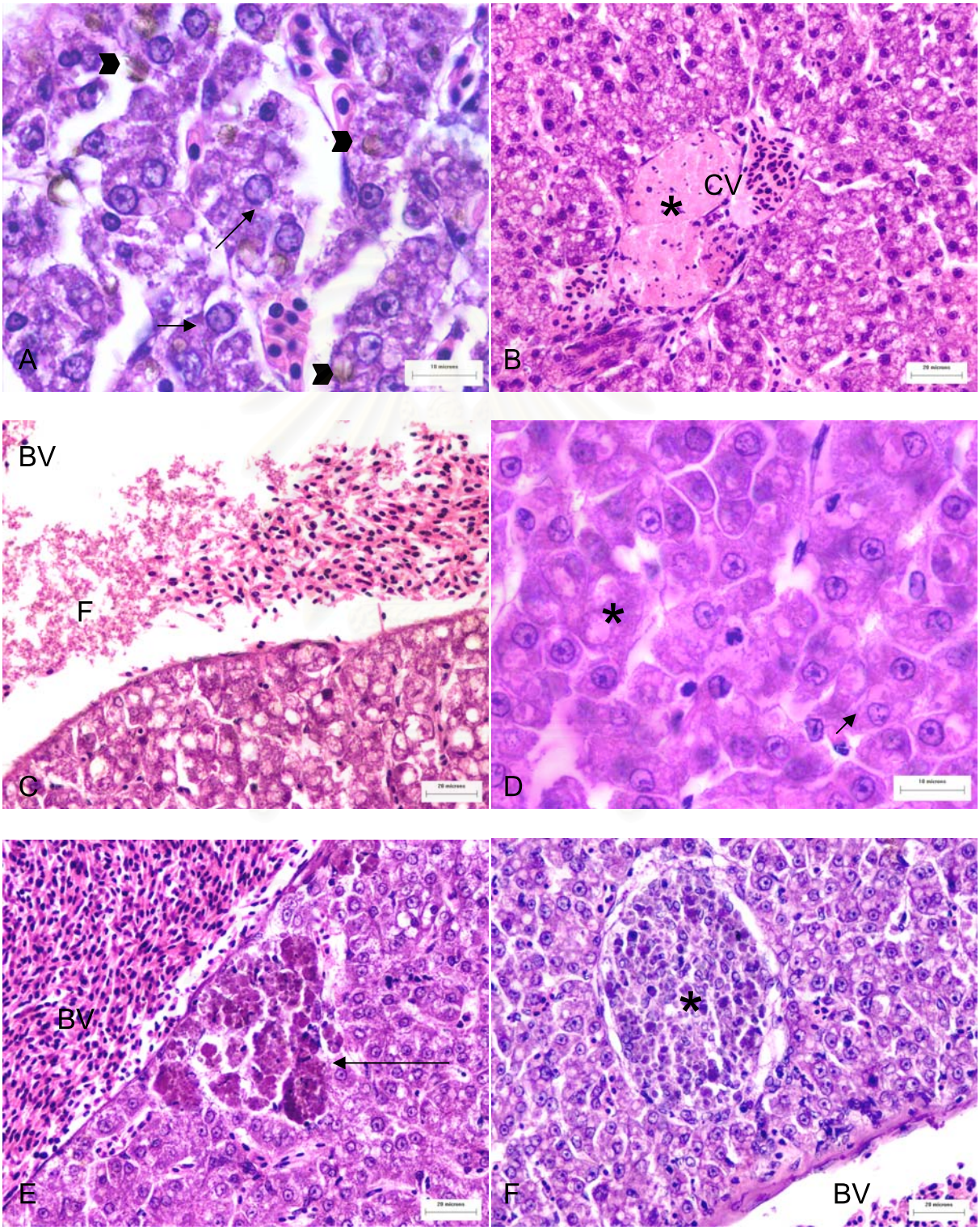

A
B
CV
BV
F
C
D
BV
E
F
BV

Plate 5

Liver of Klong 7 in rainy season fish

(H&E staining)

Figure A Photomicrograph of liver of rainy season group shows focal necrosis (*) and sinusoid dilated (→).

Figure B Photomicrograph of liver of rainy season group shows the infiltration of macrophage surround the blood vessel (→) and accumulation of hyaline granules in cytoplasm of hepatocyte (>).

Figure C Photomicrograph of liver of rainy season group shows sinusoid without erythrocyte (→). Pancreatic cell shrinkage (P).

Figure D Photomicrograph of liver of rainy season group shows blood congestion (→), and eosinophilic cytoplasm (EC).

Figure E Photomicrograph of liver of rainy season group shows accumulation of lipid droplet in cytoplasm of hepatocyte (*).

Figure F Photomicrograph of liver of rainy season group shows group of necrotic cells with fibrous capsule (→) and disarrangement of hepatic plate (*).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein, and BV: Blood vessel.

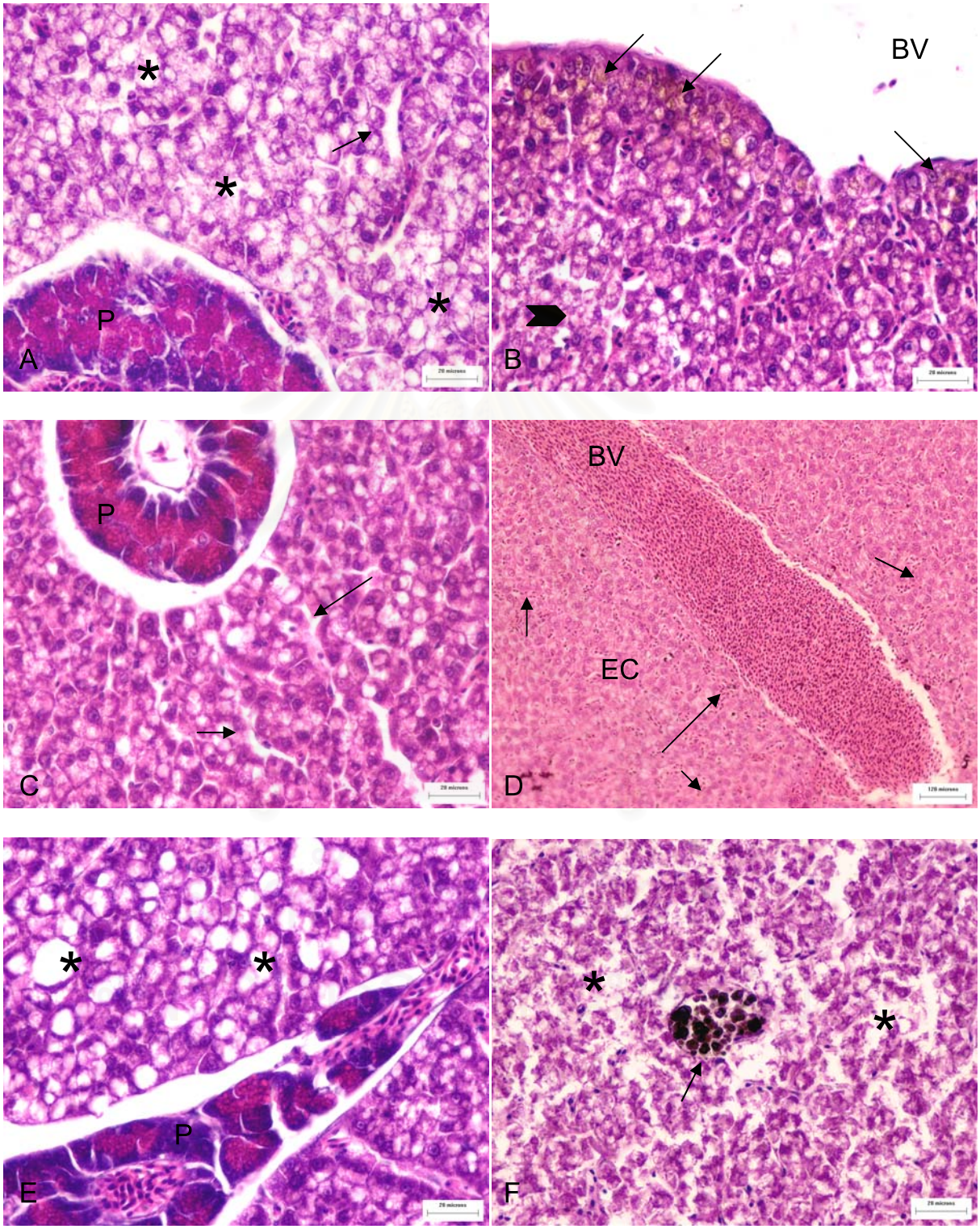

A
P
B
BV
C
P
D
BV
EC
E
P
F

Plate 6

Liver of Klong 7 in rainy season fish

(H&E staining)

Figure A Photomicrograph of liver of rainy season group shows the detachment of capsular epithelial lining (→) and diffuse necrosis (*).

Figure B Photomicrograph of liver of rainy season group shows the detachment of endothelial lining (→) and fragment of necrotic erythrocytes in blood vessel (F).

Figure C Photomicrograph of liver of rainy season group shows karyolysis nucleus (*), perinuclear chromatin clumping nucleus (>), and infiltration of macrophage (→).

Figure D Photomicrograph of liver of rainy season group shows the accumulation of lipid droplets in hepatocyte (>).

Figure E Photomicrograph of liver of rainy season group shows the infiltration of macrophage at the rim of blood vessel (→).

Figure F Photomicrograph of liver of rainy season group shows the disarrangement of hepatic plate (HP).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein,
BV: Blood vessel, and P: Pancreas

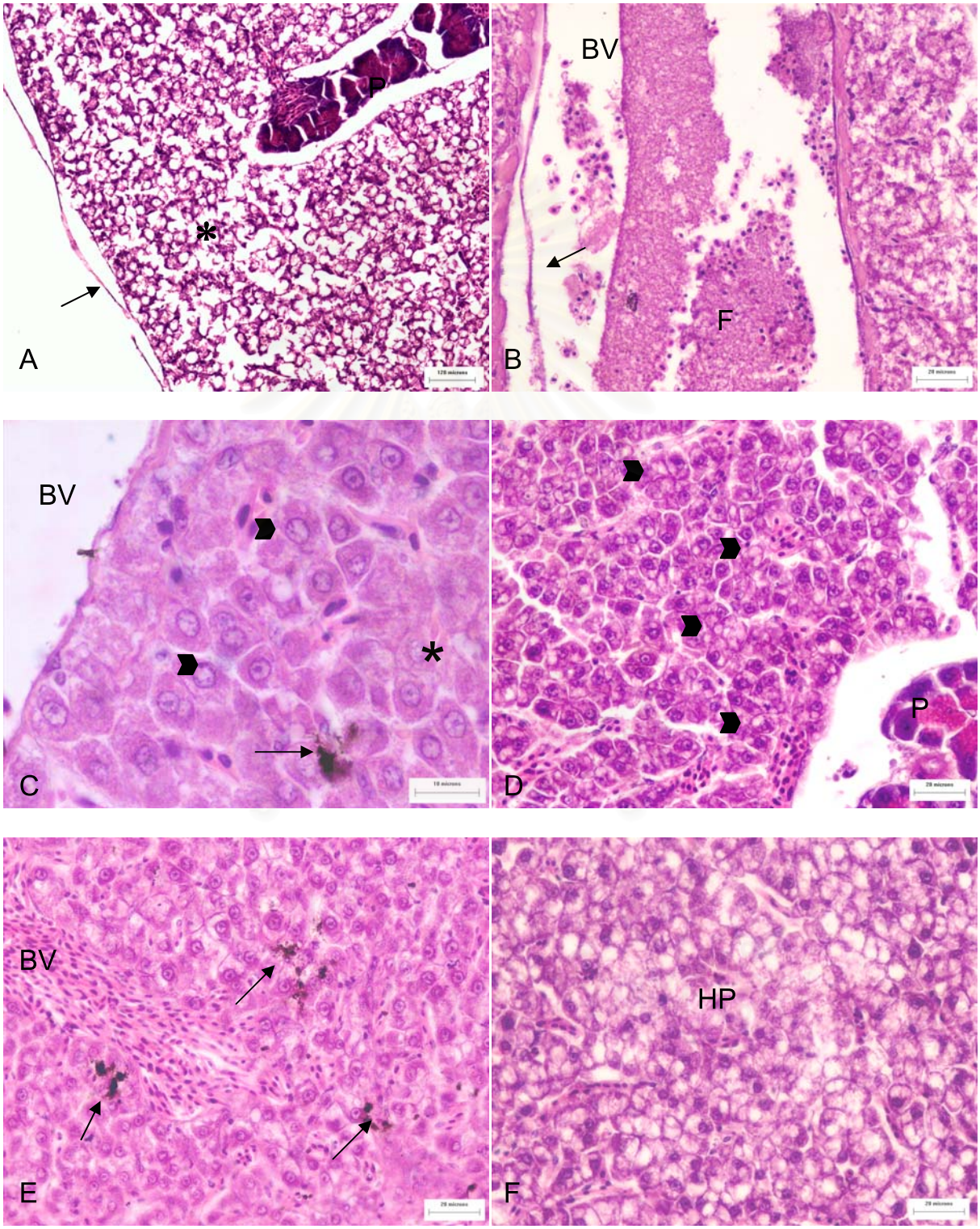

P
A
BV
F
B
BV
C
P
D
BV
E
HP
F

Plate 7

Liver of 0.01 μg/L endosulfan treated fish

(H&E staining)

Figure A Photomicrograph of treated liver shows eosinophilic cytoplasm (EC), accumulation of hyaline granule in cytoplasm (>), and pancreatic cell shrinkage (P).

Figure B Photomicrograph of treated liver shows the detachment of endothelial lining (→) and accumulation of hyaline granule in cytoplasm (>).

Figure C Photomicrograph of treated liver shows focal necrosis (*) and swelling erythrocytes in blood vessel (BV). Disarrangement of hepatic plate (HP).

Figure D Photomicrograph of treated liver shows focal necrosis (*) and perinuclear chromatin clumping nucleus (>).

Figure E Photomicrograph of treated liver shows karyolysis (*) and pyknotic nucleus (>).

Figure F Photomicrograph of treated liver shows abnormal shape of some erythrocyte (>).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein, and BV: Blood vessel.

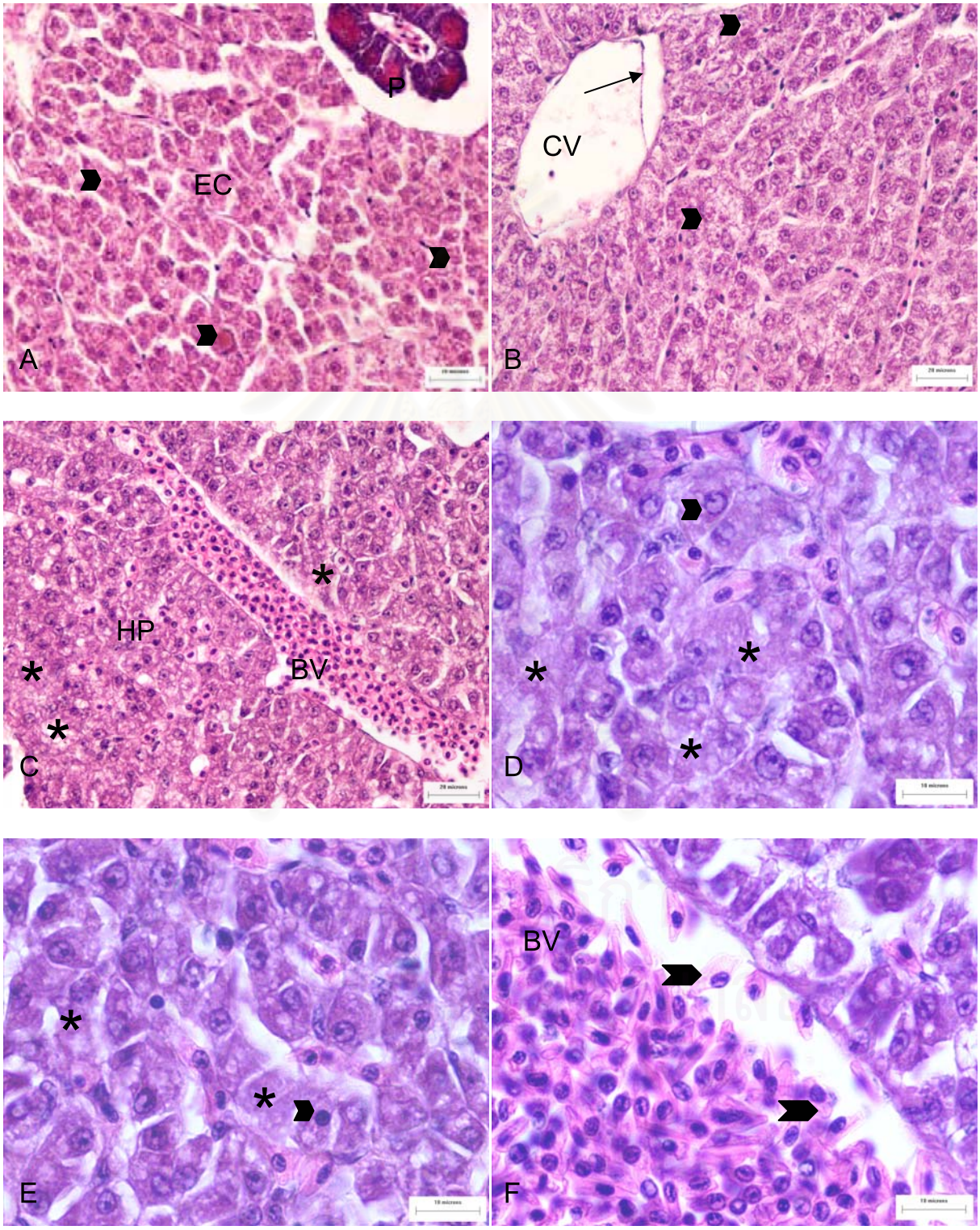

P
EC
A
CV
B
HP
BV
C
D
E
BV
F

Plate 8

Liver of 0.06 μg/L endosulfan treated fish

(H&E staining)

Figure A Photomicrograph of treated liver shows focal necrosis (*).

Figure B Photomicrograph of treated liver shows focal necrosis (>), accumulation of hyaline granule (→), and eosinophilic cytoplasm (EC). Blood vessel (BV) shows fragment of necrotic erythrocytes.

Figure C Photomicrograph of treated liver shows the detachment of capsular epithelial lining (→), accumulation of hyaline granule (>), and focal necrosis (*).

Figure D Photomicrograph of treated liver shows the accumulation of hyaline granule in hepatocyte (>), karyolysis (→), and focal necrosis (*).

Figure E Photomicrograph of treated liver shows focal necrosis (*). Pancreatic cell shrinkage (P).

Figure F Photomicrograph of treated liver shows the disarrangement of hepatic plate (HP) and pyknotic nucleus (→). Pancreatic cell shrinkage (P).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein,
BV: Blood vessel, and BD: Bile duct

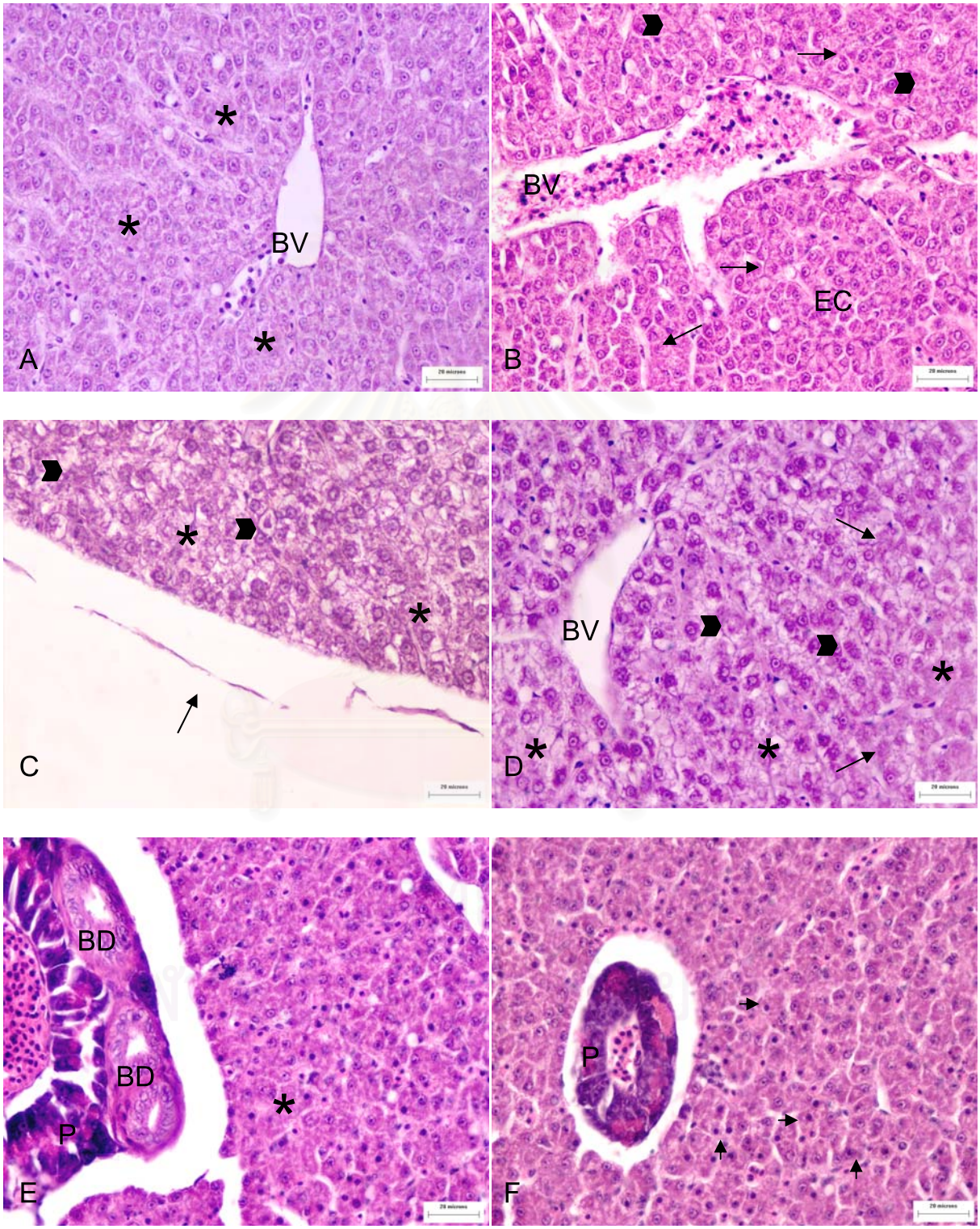

A
BV
B
BV
EC
C
D
BV
E
BD
BD
P
F
P

Plate 9

Liver of 0.5 μg/L endosulfan treated fish

(H&E staining)

Figure A Photomicrograph of treated liver shows the detachment of capsular epithelial lining (⟶), and the accumulation of hyaline granules in cytoplasm (>).

Figure B Photomicrograph of treated liver shows the detachment of endothelial lining (⟶), accumulation of hyaline granule in hepatocyte (>), and focal necrosis (*).

Figure C Photomicrograph of treated liver shows the disarrangement of hepatic plate (HP) and the accumulation of hyaline granule in hepatic cell (⟶). Pancreatic cell shrinkage (P).

Figure D Photomicrograph of treated liver shows focal necrosis (*) and the accumulation of hyaline granule in hepatocyte (>).

Figure E Photomicrograph of treated liver shows the accumulation of lipid droplet in hepatocyte (>), perinuclear chromatin clumping nucleus (⟶), and karyolysis (*). Pancreatic cell shrinkage (P).

Figure F Photomicrograph of treated liver shows focal necrosis (*) and the accumulation of hyaline granule in cytoplasm (>). Pancreatic cell shrinkage (P).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein, and BV: Blood vessel.

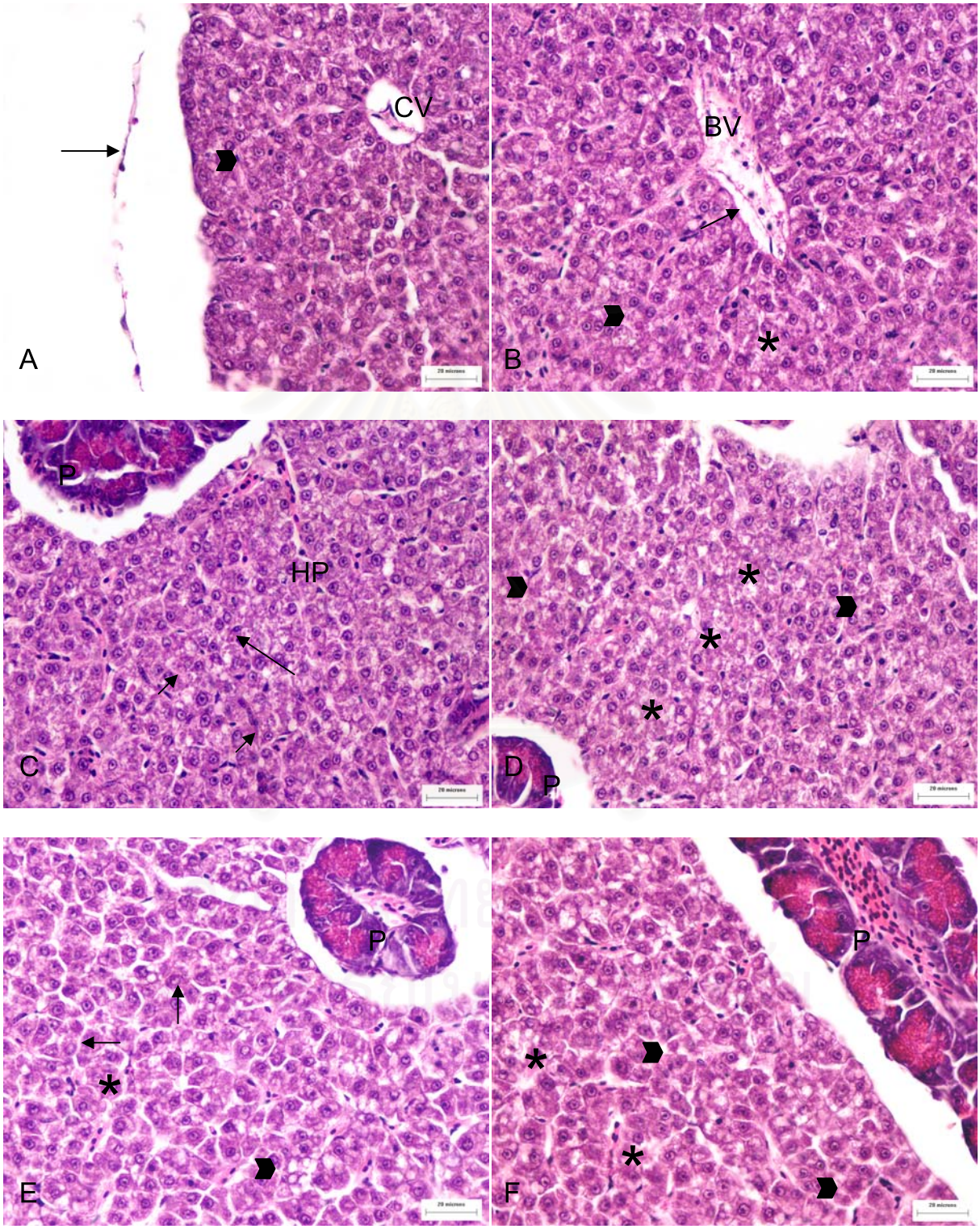

CV
BV
A
B
P
HP
C
D
P
P
E
P
F

Plate 10

Liver of 1 μg/L endosulfan treated fish

(H&E staining)

Figure A Photomicrograph of treated liver shows group of necrotic cells (⟶) and morphological alteration of erythrocytes (F).

Figure B Photomicrograph of treated liver shows the accumulation of hyaline granule in hepatocyte (>) and karyolysis nucleus (*).

Figure C Photomicrograph of treated liver shows the disarrangement of hepatic plate (HP), karyolysis (*), and accumulation of hyaline granule in hepatocyte (>).

Figure D Photomicrograph of treated liver shows the detachment of endothelial lining (⟶) and the accumulation of hyaline granule in hepatocytes (>).

Figure E Photomicrograph of treated liver shows the accumulation of large lipid droplet in hepatocyte (*) and focal necrosis (⟶). Pancreatic cell shrinkage (P).

Figure F Photomicrograph of treated liver shows focal necrosis (*), perinuclear chromatin clumping nuceus (>), and pyknotic nucleus (⟶).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein, and BV: Blood vessel.

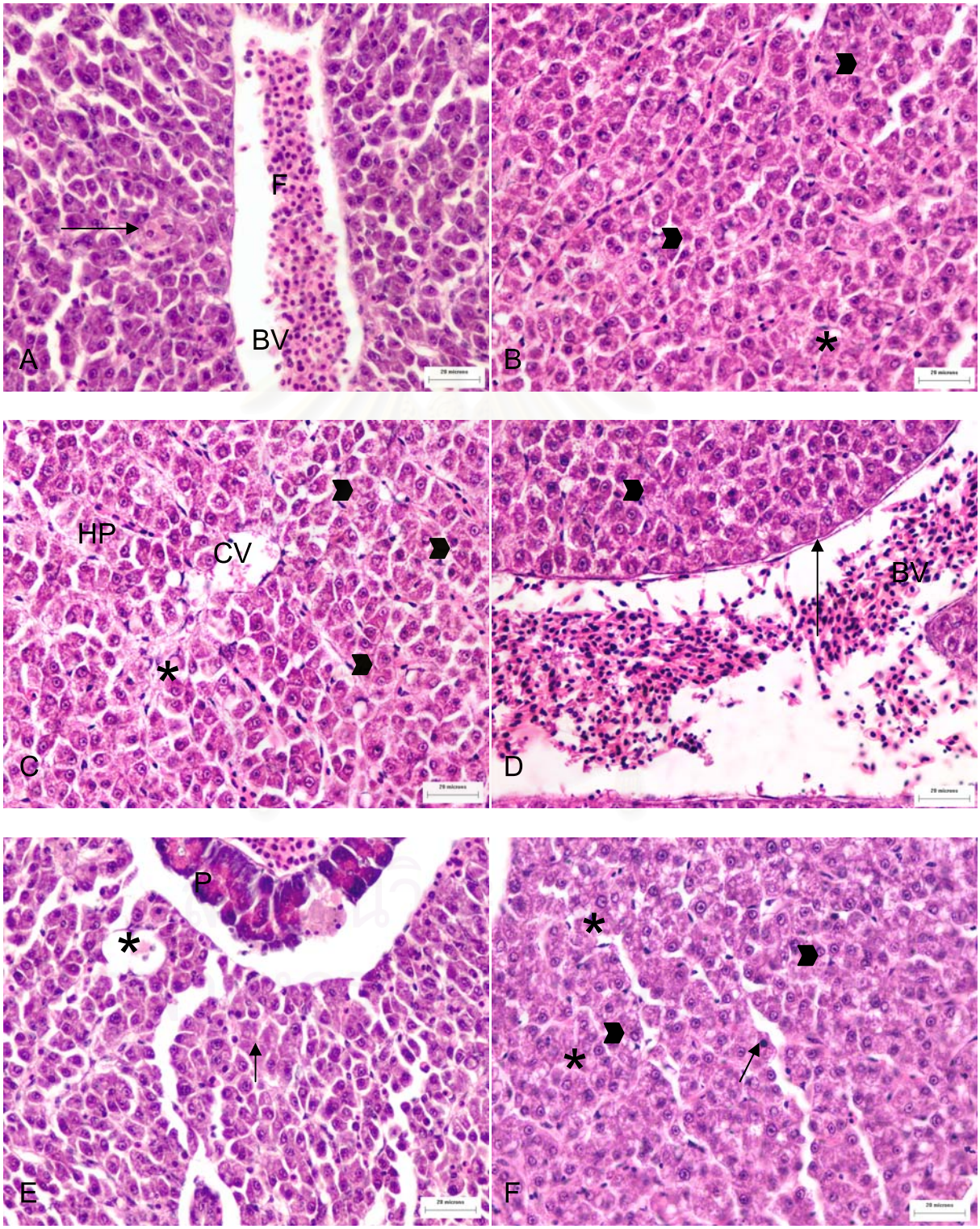

F
BV
A
B
HP
CV
C
BV
D
P
E
F

Plate 11

Comparison of liver between control, Klong 7 in summer and Klong 7 in rainy season group (H&E staining)

Figure A Photomicrograph of the control liver shows normal structure. Structure of liver composes of hepatic plate (HP). A double row of hepatocytes (H), tube is surrounded by sinusoid and most of hepatocytes nuclei are located basally to sinusoid (S).

Figure B Photomicrograph of the control liver shows normal structure hepatocyte and pancreas (P) that insert in liver parenchyma.

Figure C Photomicrograph of the summer liver shows the detachment of endothelial lining (→), diffuse necrosis (*) and the accumulation of hyaline granule in hepatic cell (>).

Figure D Photomicrograph of the summer liver shows focal necrosis (*), sinusoid dilatation (→), and the accumulation of lipid droplet in hepatic cell (>).

Figure E Photomicrograph of the rainy season liver shows the detachment of endothelial lining (→), diffuse necrosis (*), disarrangement of hepatic plate (HP), and fragment of necrotic erythrocyte in blood vessel (F).

Figure F Photomicrograph of the rainy season liver shows sinusoid dilated (→), accumulation of lipid droplet in hepatocytes (>), and focal necrosis (*).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein, BV: Blood vessel, and P: Pancreas

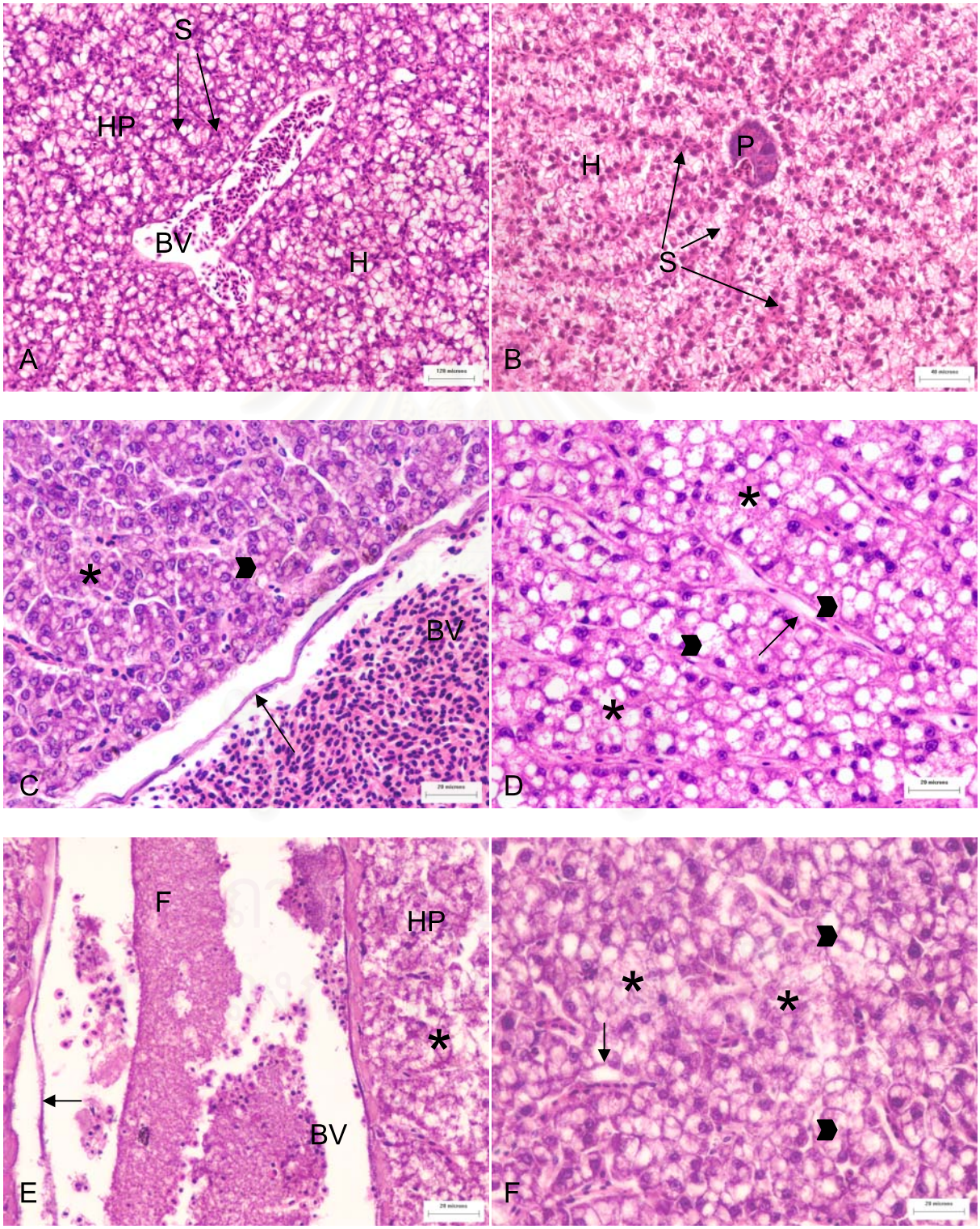

S
HP
BV
H
A
H
P
S
B
*
BV
C
*
*
D
F
HP
*
BV
E
*
*
F

Plate 12

Comparison of liver between control group and endosulfan treated fish

(H&E staining)

Figure A Photomicrograph of the control liver shows the normal structure. Structure of liver have hepatic plate (HP). A double row of hepatocytes (H), each tube is surrounded by sinusoid (S).

Figure B Photomicrograph of the control liver shows normal hepatocyte.

Figure C Photomicrograph of treated liver at 0.01 μg/L liver shows the detachment of endothelial lining (⟶) and accumulation of hyaline granule in hepatic cell (>).

Figure D Photomicrograph of treated liver at 0.06 μg/L liver shows accumulation of hyaline granule (>), karyolysis (⟶), and focal necrosis (*).

Figure E Photomicrograph of treated liver at 0.5 μg/L liver shows the accumulation of lipid droplet in hepatocyte (>), perinuclear chromatin clumping nucleus (⟶), karyolysis (*).

Figure F Photomicrograph of treated liver at 1 μg/L liver shows the disarrangement of hepatic plate (HP), karyolysis (*), and the accumulation of hyaline granule in hepatocyte (>).

HP: Hepatic plate, H: Hepatocyte, S: Sinusoid, CV: Central vein,
BV: Blood vessel, and P: Pancreas

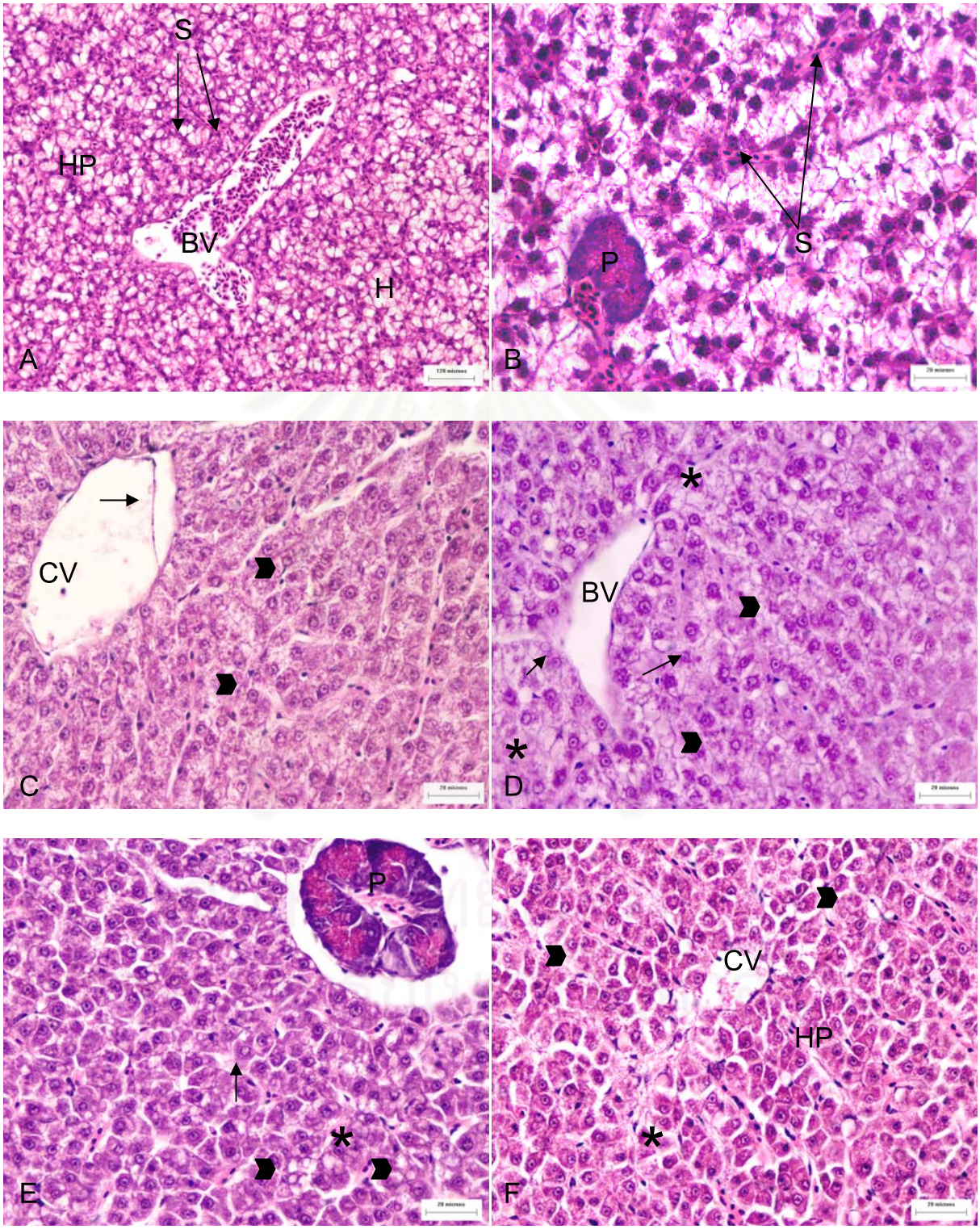

S
HP
BV
H
A
P
S
B
CV
C
BV
*
*
D
P
*
E
CV
HP
*
F

Plate 13

Comparision of liver between control, Klong 7 in summer,
Klong 7 in rainy season, and endosulfan treated group
(Oil red O staining)

Figure A Photomicrograph of the control liver shows small size lipid droplet and moderately dense of droplets deposition in hepatic parenchyma.

Figure B Photomicrograph of summer group liver shows large size lipid droplet and extremely dense of droplets deposition in hepatic parenchyma.

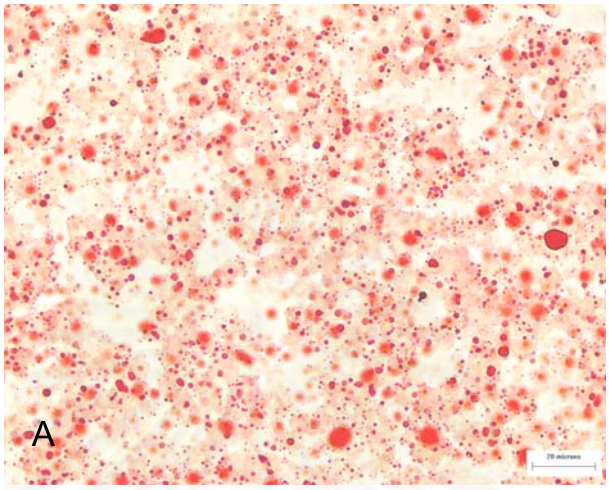
Figure C Photomicrograph of rainy season group liver shows the mixed of small and large size lipid droplet and strongly dense of droplets deposition in hepatic parenchyma.

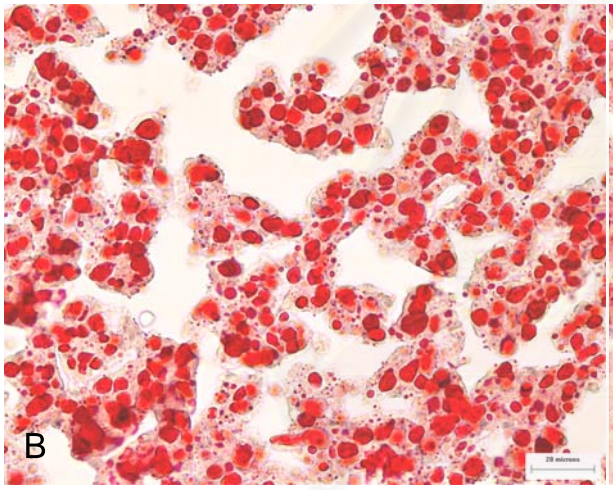
Figure D Photomicrograph of 0.01 μg/L endosulfan treated liver shows small size lipid droplet and moderately dense of droplets deposition in hepatic parenchyma.

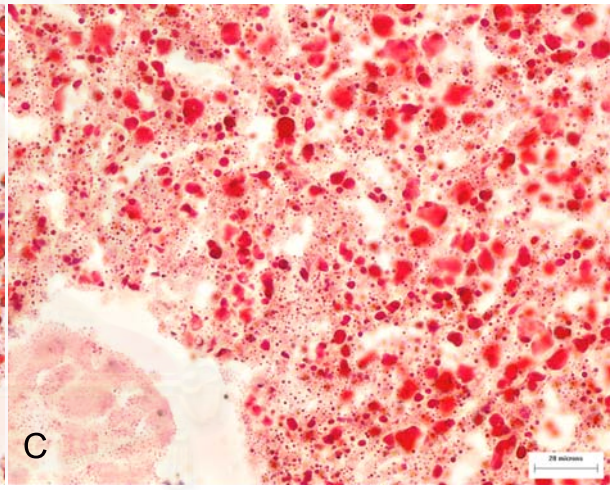
Figure E Photomicrograph of 0.06 μg/L endosulfan treated liver shows small size lipid droplet and moderately dense of droplets deposition in liver tissue.

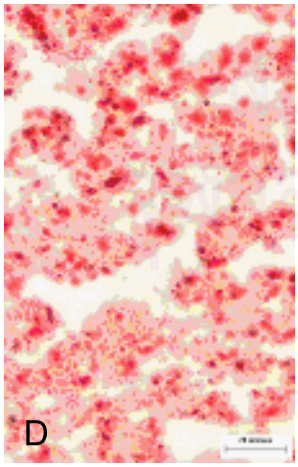
Figure F Photomicrograph of 0.5 μg/L endosulfan treated liver shows small size lipid droplet and mild dense of droplets deposition in liver.

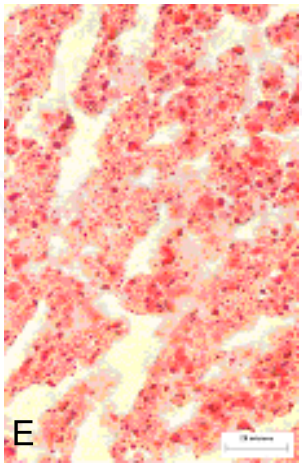
Figure G Photomicrograph of 1 μg/L endosulfan treated liver shows a very small size and mildly dense of droplets deposition in liver tissue.

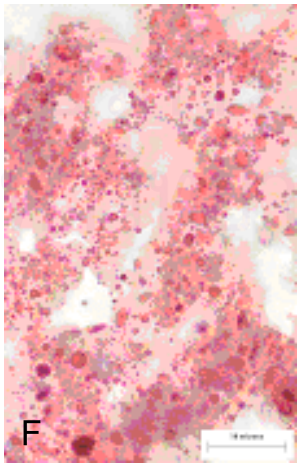

A


B


C


D


E


F

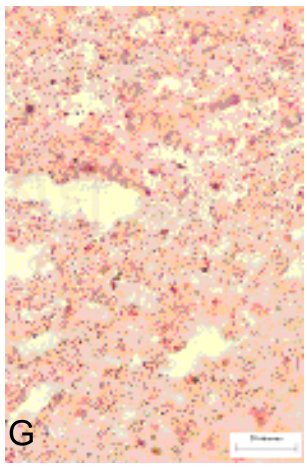

G

Plate 14

Comparision of liver between control, Klong 7 in summer,
Klong 7 in rainy season, and endosulfan treated group
(PAS staining)

Figure A Photomicrograph of the control liver shows normal deposition of glycogen in liver tissue.

Figure B Photomicrograph of summer group liver shows the moderately deposition of glycogen in liver tissue.

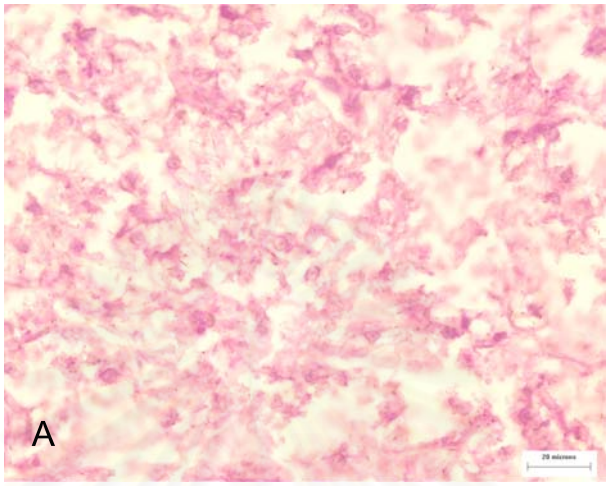
Figure C Photomicrograph of rainy season group liver shows the densely deposition of glycogen in liver tissue.

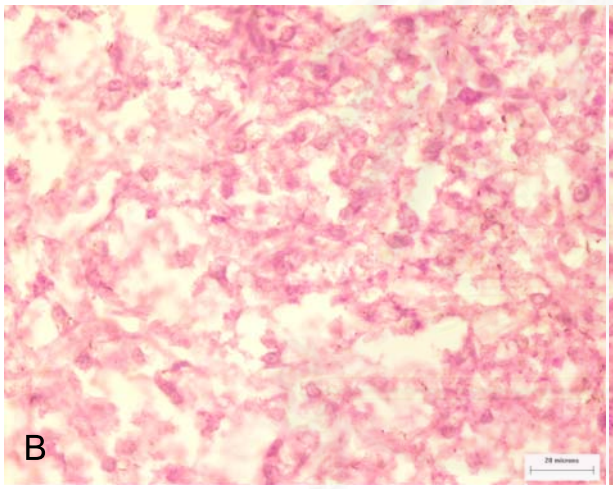
Figure D Photomicrograph of 0.01 μg/L endosulfan treated liver shows the moderately deposition of glycogen in liver tissue.

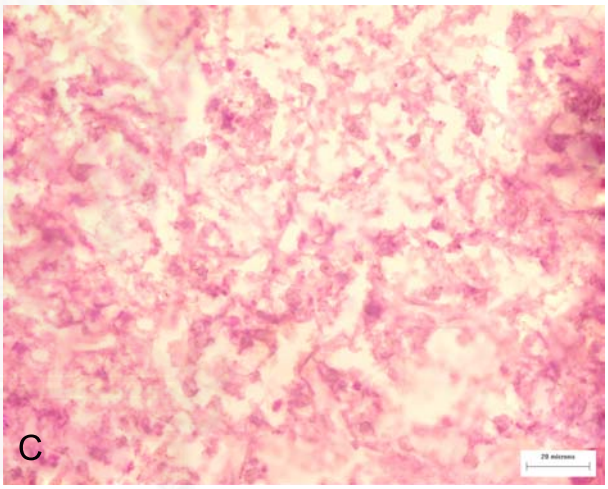
Figure E Photomicrograph of 0.06 μg/L endosulfan treated liver shows the slightly deposition of glycogen in liver tissue.

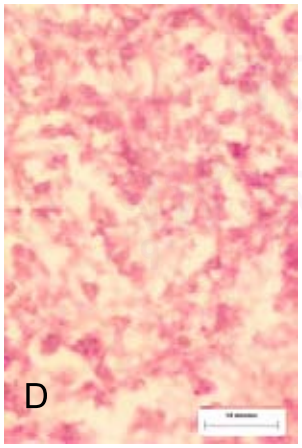
Figure F Photomicrograph of 0.5 μg/L endosulfan treated liver shows the very slight deposition of glycogen in liver tissue.

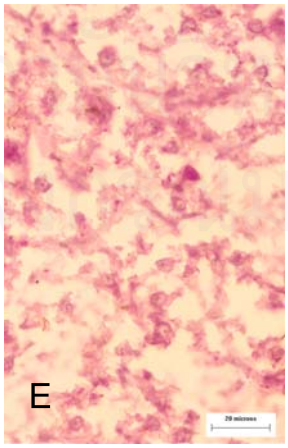
Figure G Photomicrograph of 1 μg/L endosulfan treated liver shows the very slight deposition of glycogen in liver tissue.

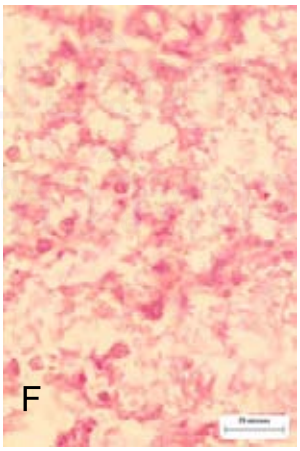

A

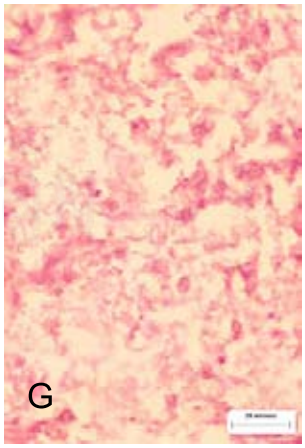

B


C


D


E


F


G

Water parameters of Klong 7, Pathum Thani Province

The water temperature, DO, and pH at stations A, B, and C were combined and recorded for mean ± SD of each month from March to November 2004. The data is shown in Table 4-3. The water temperature was highest in April at 33.4±2.3°C and lowest in September 2004 at 28.8±1.1°C (Figure 4-1). The range of DO was from 3.2±1.0 to 6.9±3.5 mg/L. DO was low in July, August, and September (Figure 4-2), which is lower than standard quality of surface water (DO = 4 mg/L) (Menasveta, 2000). This indicated that the Klong 7 had already received some amounts of organic pollutants from dead vegetations and wastes from irrigated agricultural lands. The range of pH was 6.1±0.0 – 7.0±0.2 (Figure 4-3) which is within the standard pH quality of surface water (pH 5-9) (Menasveta, 2000). Therefore, the water at Kong 7 may not have severely adverse effects on aquatic organisms.

Table 4-3 Averages of the water parameters at Klong 7 from March to Novembar 2004

	Month								
	March (Mean±SD)	April (Mean±SD)	May (Mean±SD)	June (Mean±SD)	July (Mean±SD)	August (Mean±SD)	September (Mean±SD)	October (Mean±SD)	November (Mean±SD)
Temperature (° C)	32.6±2.7	33.4±2.3	30.9±0.2	30.5±0.2	31.8±1.1	31.4±0.1	28.8±1.1	30.8±0.7	31.8±1.4
DO (mg/L)	6.5±0.9	6.9±3.5	4.0±0.3	5.0±1.2	3.2±1.2	3.6±0.2	3.2±1.0	4.3±0.4	6.1±0.7
pH	6.6±0.0	6.6±0.5	6.1±0.0	6.2±0.0	6.9±0.3	7.0±0.2	6.7±0.2	6.7±0.2	6.6±0.5

Figure 4-1 Averages of the water temperature at Klong 7 from March to November 2004

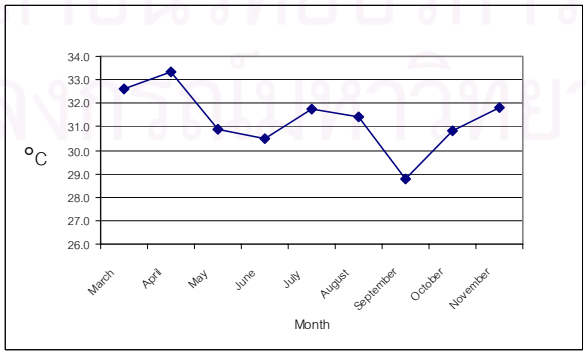


Figure 4-2 Averages of the water DO at Klong 7 from March to November 2004

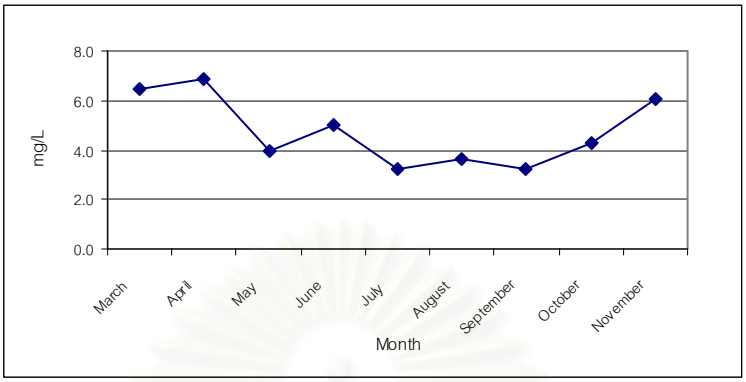
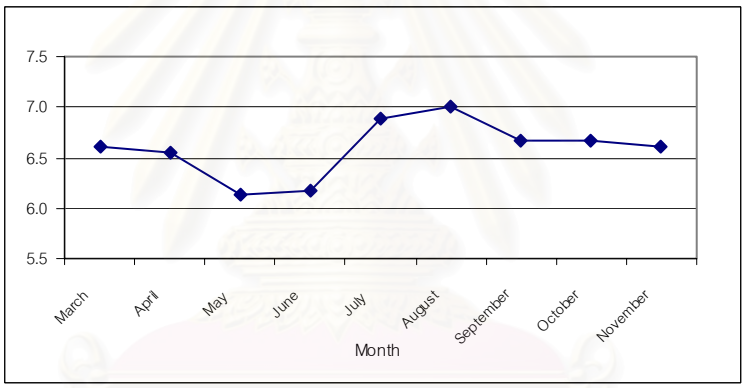


Figure 4-3 Averages of the water pH at Klong 7 from March to November 2004



Quantitative analysis of organochlorine pesticide residues in water at Klong 7, Pathum Thani Province

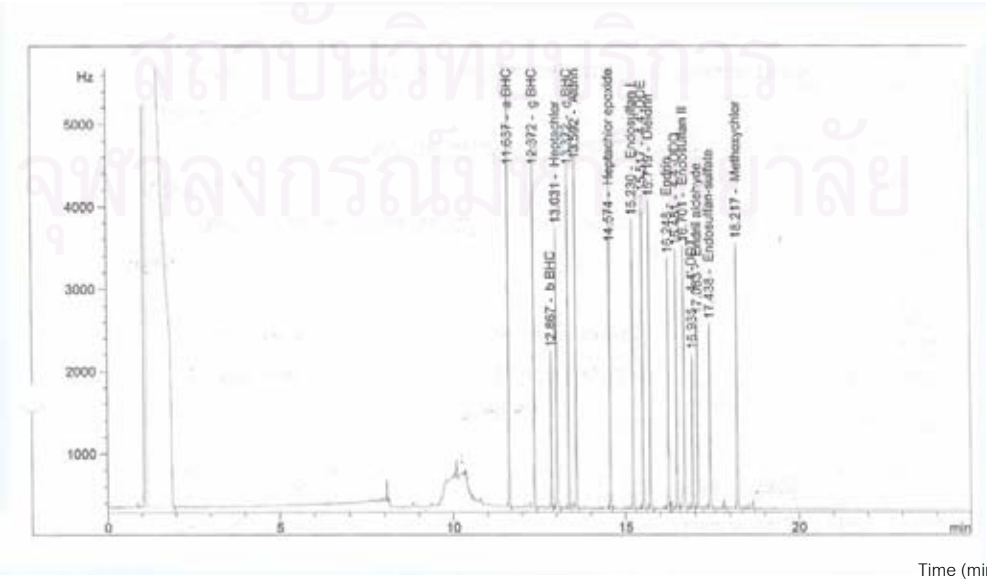
Calibration curve

The results of R^2 and retention time of the mixed OPCs standard solutions covering the concentrations of 2-50 μg/L are shown in Table 4-4 and Figure 4-4.

Table 4-4 Retention time and R^2 of the mixed standard organochlorine pesticide solutions.

No	Organochlorine pesticide	Retention Time (min)	R^2
1	α BHC	11.58	0.9998
2	γ BHC	12.32	0.9995
3	β BHC	12.82	0.9997
4	Heptachlor	12.95	0.9994
5	δ BHC	13.32	0.9991
6	Aldrin	13.54	0.9993
7	Heptachlor epoxide	14.52	0.9995
8	Endosulfan I	15.18	0.9998
9	4-4' DDE	15.47	0.9986
10	Dieldrin	15.67	0.9991
11	Endrin	16.20	0.9992
12	4-4' DDD	16.43	0.9992
13	Endosulfan II	16.65	0.9993
14	4-4' DDT	16.89	0.9996
15	Endrin aldehyde	17.03	0.9991
16	Endosulfan sulfate	17.38	0.9993
17	Methoxychlor	18.15	0.9984

Figure 4-4 Retention time and elution order of mixed 17 standard organochlorine pesticide solutions at the concentration 5 μg/L



Quality control (QC)

Blank

From figure 4-5 the results indicated that the presence of organochlorine pesticides was not found in the blank. Therefore, this method was used with confidence.

Field replicate

α BHC

The range concentrations of α BHC from March, April, May, June, July, August, September, October, November, and December 2004 were <0.028 – 4.44 ng/L , <0.028 ng/L, <0.028 – 4.14 ng/L, <0.028 – 4.32 ng/L, <0.028 – 4.46 ng/L, <0.028 – 1.91 ng/L, <0.028 – 16.70 ng/L, <0.028 – 2.20 ng/L, 2.01 – 2.92 ng/L, and <0.028 – 2.56 ng/L, respectively.

γ BHC

The range concentrations of γ BHC from March, April, May, June, July, August, September, October, November, and December 2004 were <0.03 – 8.33 ng/L, 4.21 – 40.90 ng/L, 4.40 – 6.08 ng/L, 4.56 – 10.16 ng/L, 3.97 – 9.48 ng/L, <0.03 – 14.83 ng/L, <0.03 – 14.58 ng/L, 2.36 – 6.00 ng/L, 2.69 – 5.67 ng/L, and 2.89 – 4.44 ng/L, respectively.

β BHC

The range concentrations of β BHC from March, April, May, June, July, August, September, October, November, and December 2004 were < 0.178 – 6.41 ng/L, <0.178 ng/L, <0.178 ng/L, <0.178 – 3.63 ng/L, <0.178 ng/L, <0.178 – 4.61 ng/L, 1.05 – 1.84 ng/L, <0.178 – 14.91 ng/L, <0.178 ng/L, and <0.178 ng/L, respectively.

Heptachlor

The range concentrations of heptachlor from March, April, May, June, July, August, September, October, November, and December 2004 were <0.006 – 6.71 ng/L,

<0.006 – 77.51 ng/L, <0.006 – 68.52 ng/L, <0.006 – 10.16 ng/L, <0.006 – 12.03 ng/L, <0.006 – 1.86 ng/L, 3.03 – 6.38 ng/L, 3.60 – 961.72 ng/L, 11.34 – 25.67 ng/L, and < 0.006 ng/L, respectively.

δ BHC

The range concentrations of δ BHC from March, April, May, June, July, August, September, October, November, and December 2004 were <0.05 - ng/L, <0.05 ng/L, <0.05 ng/L, <0.05 ng/L, <0.05 – 5.11 ng/L, < 0.05 – 3.54 ng/L, <0.05 – 14.30 ng/L, <0.05 ng/L, <0.05 ng/L, and <0.05 – 2.32 ng/L, respectively.

Aldrin

The range concentrations of aldrin from March, April, May, June, July, August, September, October, November, and December 2004 were <0.025 – 5.02 ng/L, 3.33 – 4.43 ng/L, <0.025 – 34.98 ng/L, <0.025 – 3.44 ng/L, 3.28 – 3.85 ng/L, <0.025 – 1.27 ng/L, <0.025 – 1.74 ng/L, 1.59 – 5.27 ng/L, 1.60 – 2.05 ng/L, and <0.025 – 15.03 ng/L, respectively.

Heptachlor epoxide

The range concentrations of heptachlor epoxide from March, April, May, June, July, August, September, October, November, and December 2004 were <0.035 ng/L, <0.035 ng/L, <0.035 ng/L, <0.035 ng/L, <0.035 ng/L, <0.035 – 1.79 ng/L, <0.035 – 1.31 ng/L, <0.035 – 8.51 ng/L, <0.035 – 9.25 ng/L, and <0.035 – 1.20 ng/L, respectively.

Endosulfan I

The range concentrations of endosulfan I from March, April, May, June, July, August, September, October, November, and December 2004 were <0.07 – 2.94 ng/L, <0.07 – 3.58 ng/L, < 0.07 – 3.21 ng/L, <0.07 – 5.83 ng/L, 3.04 – 43.00 ng/L, 2.33 – 62.16 ng/L, 0.61 – 10.59 ng/L, 1.55 – 8.24 ng/L, 1.15 – 2.25 ng/L, and 1.13 – 2.75 ng/L, respectively.

4, 4'-DDE

The range concentrations of 4, 4'-DDE from March, April, May, June, July, August, September, October, November, and December 2004 were <0.04 ng/L, <0.04 – 4.62 ng/L, <0.04 ng/L, <0.04 – 3.98 ng/L, <0.04 – 4.05 ng/L, <0.04 ng/L, <0.04 ng/L, <0.04 ng/L, 0.04 ng/L, and <0.04 – 1.78 ng/L, respectively.

Dieldrin

The range concentrations of dieldrin from March, April, May, June, July, August, September, October, November, and December 2004 were 2.86 – 7.77 ng/L, <0.02 – 4.82 ng/L, <0.02 – 4.64 ng/L, <0.02 – 4.77 ng/L, < 0.02 – 4.97 ng/L, 1.13 – 2.78 ng/L, 1.15 – 3.76 ng/L, <0.02 – 4.47 ng/L, <0.02 – 3.09 ng/L, and < 0.02 – 7.79 ng/L, respectively.

Endrin

The range concentrations of endrin from March, April, May, June, July, August, September, October, November, and December 2004 were <0.10 – 4.70 ng/L, <0.10 ng/L, <0.10 ng/L, <0.10 – 4.38 ng/L, 3.9 – 42.72 ng/L, 1.52 – 3.84 ng/L, <0.10 – 3.38 ng/L, <0.10 – 3.65 ng/L, <0.10 – 3.75 ng/L, and 1.49 – 4.69 ng/L, respectively.

4, 4'-DDD

The range concentrations of 4, 4'-DDD from March, April, May, June, July, August, September, October, November, and December 2004 were <0.09 ng/L, <0.09 ng/L, <0.09 ng/L, <0.09 ng/L, <0.09 ng/L, <0.09 ng/L, <0.09 – 9.81 ng/L, <0.09 – 10.43 ng/L, <0.09 – 0.77 ng/L, and <0.09 – 1.22 ng/L, respectively.

Endosulfan II

The range concentrations of endosulfan II from March, April, May, June, July, August, September, October, November, and December 2004 were <0.008 – 4.97 ng/L, <0.008 – 4.10 ng/L, <0.008 – 2.92 ng/L, <0.008 – 27.63 ng/L, <0.008 – 5.26 ng/L, 1.45 – 66.91 ng/L, <0.08 – 12.82 ng/L, 1.16 – 18.61 ng/L, <0.008 – 10.33 ng/L, and < 0.008 – 6.78 ng/L, respectively.

4, 4'-DDT

The range concentrations of 4, 4'-DDT from March, April, May, June, July, August, September, October, November, and December 2004 were 18.03 – 26.19 ng/L, 9.55 – 17.00 ng/L, 1.36 – 15.86 ng/L, 14.51 – 27.83 ng/L, 17.78 – 35.37 ng/L, 10.40 – 15.54 ng/L, 6.38 – 47.82 ng/L, 19.50 – 51.60 ng/L, 1.87 – 19.23 ng/L, and 12.04 – 36.62 ng/L, respectively.

Endrin aldehyde

The range concentrations of endrin aldehyde from March, April, May, June, July, August, September, October, November, and December 2004 were <0.08 ng/L, <0.08 ng/L, <0.08 – 3.83 ng/L, <0.08 ng/L, <0.08 ng/L, <0.08 ng/L, <0.08 ng/L, <0.08 – 5.95 ng/L, <0.08 – 32.40 ng/L, and <0.08 – 2.24 ng/L, respectively.

Endosulfan sulfate

The range concentrations of endosulfan sulfate from March, April, May, June, July, August, September, October, November, and December 2004 were 9.28 – 31.92 ng/L, 8.04 – 30.68 ng/L, 8.39 – 19.23 ng/L, 28.57 – 61.83 ng/L, 30.31 – 45.00 ng/L, 23.258 – 618.70 ng/L, 11.897 – 21.554 ng/L, 6.58 – 19.25 ng/L, 29.26 – 39.78 ng/L, and 10.79 – 38.06 ng/L, respectively.

Methoxychlor

The range concentrations of methoxychlor from March, April, May, June, July, August, September, October, November, and December 2004 were <0.167 ng/L, 6.27 – 6.74 ng/L, <0.167 ng/L, <0.167 ng/L, <0.167 ng/L,<0.167 – 1.31 ng/L, <0.167 – 1.31 ng/L, <0.167 ng/L, <0.167 ng/L, and < 0.167 – 4.60 ng/L, respectively.

The percentage of recovery

The percentage of recovery is used to assess the accuracy and the extraction efficiency. For this study, the percentage recoveries were obtained at 80-130, which is an acceptable range 70-130% of the extraction (Massachusetts department of Environmental Protection, 2002) (Table 4-6).

Limit of Detection (LOD) and Limit of Quantitation (LOQ) of GC chromatography micro Electron Capture

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were defined as the peak height of analyte in mixed standard solutions that signaled significantly different from the peak height of noise equal to 3 for LOD and 10 for LOQ of each compound. These are shown in Table 4-5.

The limit of detection and limit of quantitation were 0.006 – 0.187 ng/L and 0.021 – 0.625 ng/L, respectively.

Table 4-5 Limit of detection (LOD) and Limit of quatitation (LOQ) of each pesticide in mixed standard solutions of organochlorine pesticides

No	Organochlorine pesticide	LOD (ng/L)	LOQ (ng/L)
1	α BHC	0.028	0.094
2	γ BHC	0.030	1.025
3	β BHC	0.178	0.595
4	Heptachlor	0.060	0.021
5	δ BHC	0.050	0.142
6	Aldrin	0.025	0.085
7	Heptachlor epoxide	0.035	0.115
8	Endosulfan I	0.070	0.242
9	4-4' DDE	0.042	0.140
10	Dieldrin	0.027	0.099
11	Endrin	0.105	0.352
12	4-4' DDD	0.092	0.307
13	Endosulfan II	0.008	0.027
14	4-4' DDT	0.014	0.047
15	Endrin aldehyde	0.081	0.275
16	Endosulfan sulfate	0.187	0.625
17	Methoxychlor	0.167	0.555

Figure 4-5 Chromatogram of blank, sample, and standard organochlorine pesticides (5 μg/L).

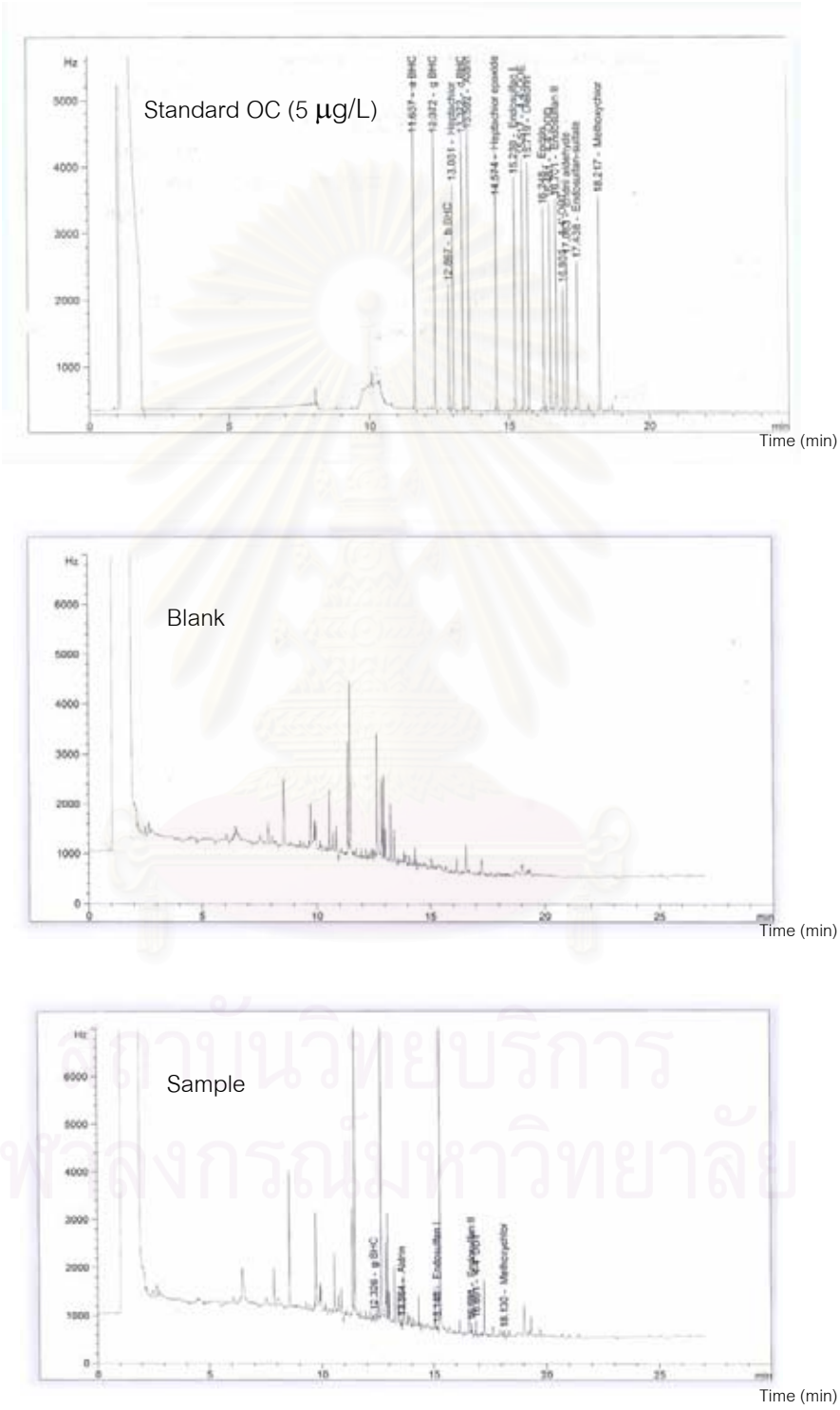


Table 4-6 Percentage recovery of the mixed organochlorine pesticides standard at final level as 5 μg/L distilled water

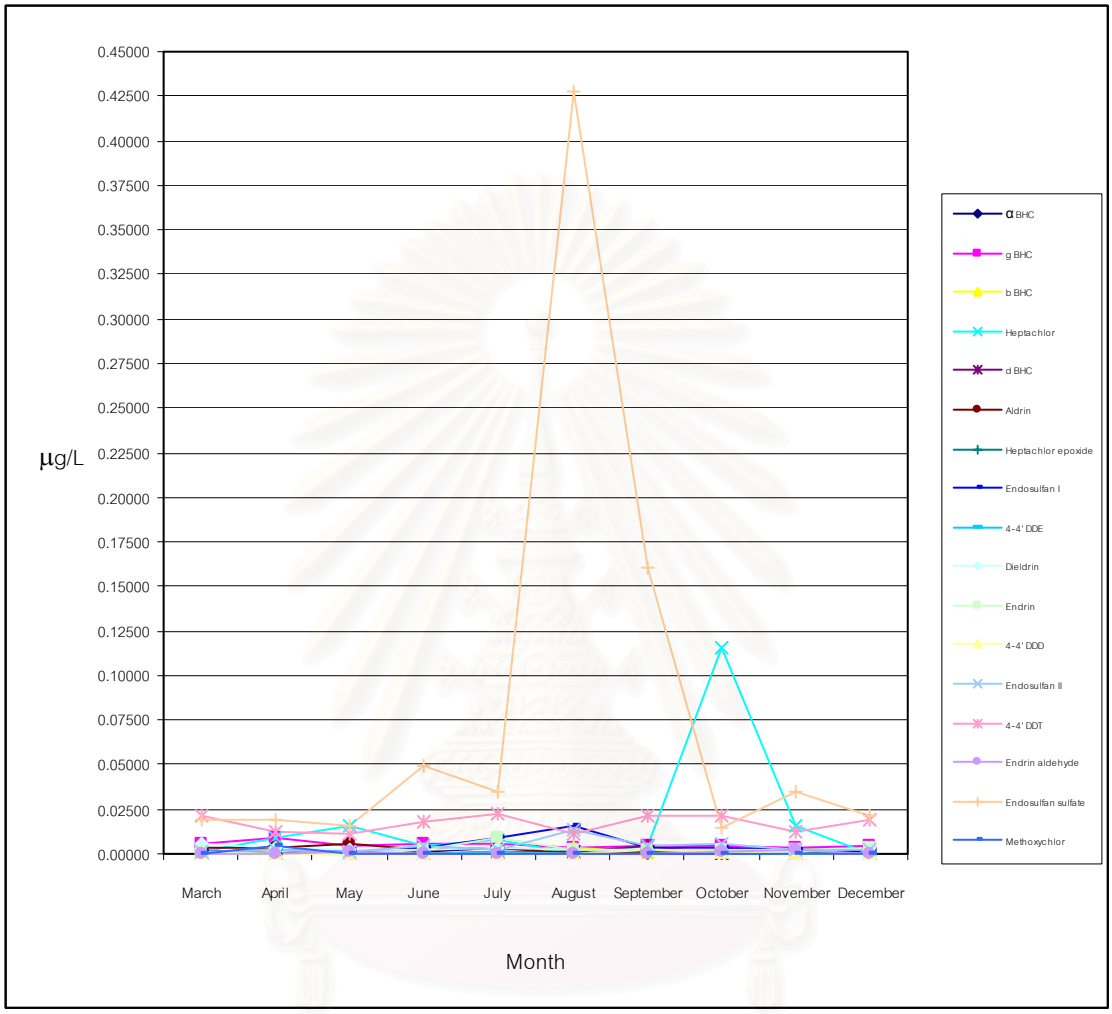
	Organochlorine pesticide								Mean	SD	%recovery	%RSD
1	α BHC	4.74	4.79	4.68	4.72	3.98	4.65	3.95	4.50	0.37	90	8
2	γ BHC	4.92	5.01	4.98	5.04	4.31	4.05	4.34	4.66	0.41	93	9
3	β BHC	5.04	5.11	5.09	5.12	5.07	4.72	5.01	5.02	0.13	101	3
4	Heptachlor	6.76	6.88	6.85	6.38	5.72	6.98	5.94	6.50	0.49	130	8
5	δ BHC	6.28	6.38	6.25	6.50	6.22	6.07	6.21	6.27	0.13	126	2
6	Aldrin	3.92	3.74	3.87	4.15	4.29	3.93	4.14	4.01	0.19	80	5
7	Heptachlor epoxide	4.45	4.50	3.94	3.96	4.45	4.31	4.60	4.32	0.26	86	6
8	Endosulfan I	4.71	3.61	3.83	4.75	4.57	4.27	5.48	4.46	0.62	89	14
9	4-4' DDE	3.74	3.81	3.93	3.86	4.04	4.61	4.36	4.05	0.32	81	8
10	Dieldrin	3.92	4.15	5.52	4.13	4.30	5.01	5.69	4.67	0.72	94	15
11	Endril	4.56	4.59	3.73	3.74	4.12	4.29	4.20	4.18	0.34	84	8
12	4-4' DDD	6.26	6.31	6.14	6.23	5.91	5.96	5.65	6.06	0.23	121	4
13	Endosulfan II	4.53	4.57	4.25	4.32	4.91	4.23	4.83	4.52	0.27	91	6
14	4-4' DDT	4.23	4.29	4.77	4.92	4.38	4.29	4.83	4.53	0.29	91	7
15	Endril aldehyde	5.64	5.61	5.85	5.91	5.48	5.98	5.72	5.74	0.18	115	3
16	Endosulfan sulfate	6.38	6.31	6.88	6.72	5.96	6.87	6.50	6.52	0.33	130	5
17	Methoxychlor	5.72	5.93	5.99	5.14	4.78	5.07	5.84	5.50	0.48	110	9

Table 4-7 Concentrations of organochlorine pesticides in the water samples collected on March to December 2004 at Klong 7, Pathum Thani Province (ng/L).

No	Pesticides	March		April		May		June		July		August		September		October		November		December	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	α BHC	1.82	0.85	ND	ND	2.63	2.28	1.33	2.31	3.75	0.84	0.99	0.71	2.77	3.46	1.18	1.09	2.21	0.12	1.29	1.25
2	γ BHC	5.93	1.15	8.74	7.18	4.94	0.12	6.13	0.57	5.56	0.62	3.37	3.45	4.00	1.54	4.14	0.71	3.89	0.92	4.01	0.92
3	β BHC	1.10	1.07	ND	ND	ND	ND	0.40	0.70	ND	ND	2.96	0.89	1.46	0.11	2.65	3.00	ND	ND	ND	ND
4	Heptachlor	1.42	2.45	9.25	14.39	15.23	13.19	4.24	3.27	8.00	1.31	0.21	0.36	4.77	1.15	115.72	187.12	15.86	2.04	ND	ND
5	δ BHC	ND	ND	ND	ND	ND	ND	ND	ND	0.57	0.98	0.69	0.62	4.62	7.24	ND	ND	ND	ND	0.26	0.45
6	Aldrin	3.18	1.68	3.74	0.49	5.55	7.28	2.53	1.33	2.50	0.19	0.55	0.23	0.97	0.84	2.41	0.83	1.78	0.01	3.37	2.32
7	Heptachlor epoxide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.35	0.31	0.15	0.25	1.30	2.25	1.14	1.69	ND	ND
8	Endosulfan I	1.54	1.42	1.89	1.37	1.36	1.18	3.46	1.78	9.03	8.00	15.26	10.78	3.68	2.86	3.01	1.08	1.70	0.25	1.70	0.48
9	4-4' DDE	ND	ND	2.80	1.47	ND	ND	0.44	0.77	1.32	2.29	ND	ND	ND	ND	ND	ND	ND	ND	0.39	0.67
10	Dieldrin	5.81	0.86	3.36	1.05	2.28	1.27	1.74	2.21	3.10	1.89	2.07	0.52	2.23	1.03	0.87	1.14	1.26	0.35	2.85	1.00
11	Endrin	0.99	1.72	ND	ND	ND	ND	1.77	1.94	8.69	7.11	2.79	0.42	2.26	0.63	1.69	1.50	2.25	0.95	2.82	1.02
12	4-4' DDD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.93	2.59	1.16	2.01	0.09	0.15	0.14	0.23
13	Endosulfan II	1.66	2.11	2.05	1.99	0.65	0.56	4.73	5.14	2.77	1.65	14.01	10.92	4.99	5.46	5.22	4.00	2.75	2.45	1.85	1.27
14	4-4' DDT	21.18	0.96	12.35	0.54	11.51	0.95	18.49	3.27	22.84	2.34	11.76	0.56	21.49	13.63	21.84	9.04	11.81	2.31	19.40	7.81
15	Endrin aldehyde	ND	ND	ND	ND	1.52	0.90	ND	ND	ND	ND	ND	ND	ND	ND	1.64	1.42	2.36	2.99	0.25	0.43
16	Endosulfan sulfate	18.73	9.59	18.77	9.28	15.19	5.48	49.48	17.61	34.41	5.41	427.8	79.63	160.48	22.91	14.11	6.00	34.28	3.23	21.23	11.54
17	Methoxychlor	ND	ND	4.32	3.74	ND	ND	ND	ND	ND	ND	0.17	0.25	0.17	0.25	ND	ND	ND	ND	0.51	0.89

ND = non detected

Figure 4-6 Concentration of each organochlorine pesticide on March to December 2004



Organochlorine pesticides in water at Klong7, Pathum Thani Province

The concentration of α BHC, γ BHC, β BHC, heptachlor, δ BHC, aldrin, heptachlor epoxide, endosulfan I, 4-4' DDE, dieldrin, endrin, 4-4' DDD, endosulfan II, 4-4' DDT, endrin aldehyde, endosulfan sulfate, and methoxychlor in water samples collected on March to December 2004 are shown in Table 4-7 and Figure 4-6. The variations of the total concentration of each organochlorine pesticide from March to December 2004 are showed in Figure 4-7 to 4-23.

α BHC

The range concentrations of α BHC from March to December 2004 were <0.028 – 3.75 ng/L (Table 4-7). The highest concentration of α BHC was used in July (3.75 ± 0.84 ng/L) while the lowest concentration was in April (<0.028 ng/L) (Figure 4-7).

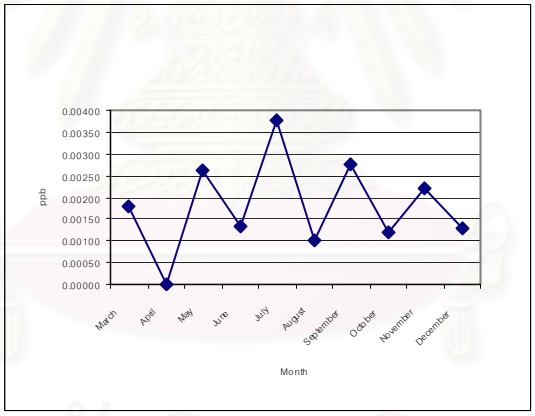


Figure 4-7 Concentrations of α BHC in Klong 7 water during March to December 2004

γ BHC

The range concentrations of γ BHC from March to December 2004 were 3.37 – 8.74 ng/L (Table 4-7). The highest concentration of γ BHC was used in April (8.74 ± 7.28 ng/L), while the lowest concentration was in August (3.37 ± 3.45 ng/L) (Figure 4-8).

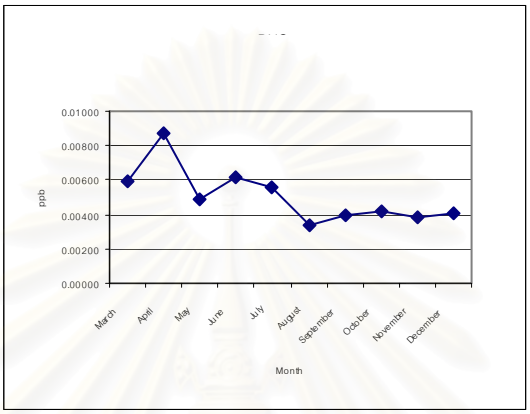


Figure 4-8 Concentrations of γ BHC in Klong 7 water during March to December 2004

β BHC

The range concentrations of β BHC from March to December 2004 were <0.178 – 2.96 ng/L (Table 4-7). The highest concentration of β BHC was used in August (2.96 ± 0.89 ng/L), while the lowest concentration was in April, May, July, November, and December (<0.178 ng/L) (Figure 4-9).

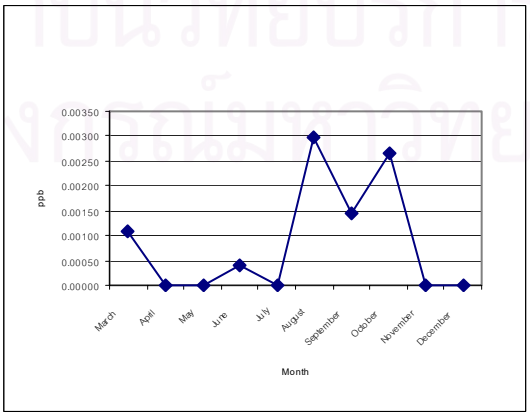


Figure 4-9 Concentrations of β BHC in Klong 7 water during March to December 2004

Heptachlor

The range concentrations of heptachlor from March to December 2004 were <0.006 – 115.72 ng/L (Table 4-7). The highest concentration of heptachlor was used in October (115.72 ± 187.12 ng/L), while the lowest concentration was in December (<0.06 ng/L) (Figure 4-10).

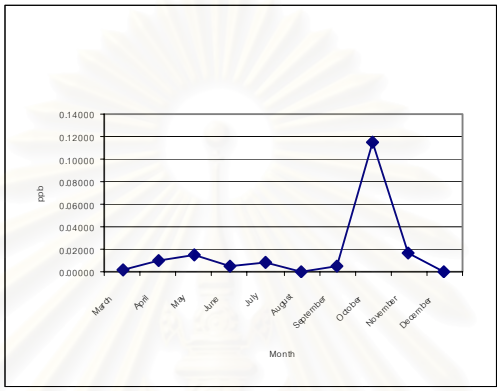


Figure 4-10 Concentrations of heptachlor in Klong 7 water during March to December 2004

δ BHC

The range concentrations of δ BHC from March to December 2004 were <0.05 – 4.62 ng/L (Table 4-7). The highest concentration of δ BHC was used in September (4.62 ± 7.24 ng/L), while the lowest concentration was in March, April, May, June, October, and November (<0.05 ng/L) (Figure 4-11).

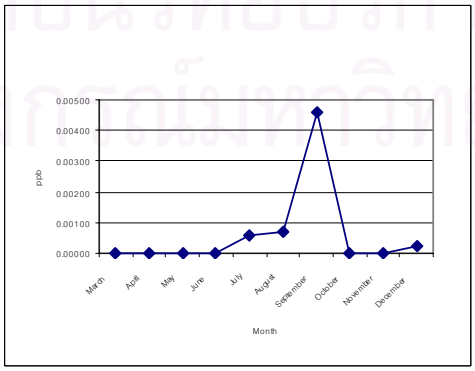


Figure 4-11 Concentrations of δ BHC in Klong 7 water during March to December 2004

Aldrin

The range concentrations of aldrin from March to December 2004 were 0.55 – 5.55 ng/L (Table 4-7). The highest concentration of aldrin was used in May (5.55 ± 7.28 ng/L), while the lowest concentration was in August (0.55 ± 0.23 ng/L) (Figure 4-12).

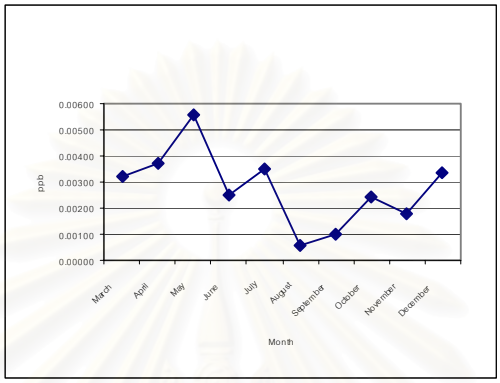


Figure 4-12 Concentrations of aldrin in Klong 7 water during March to December 2004

Heptachlor epoxide

The range concentrations of heptachlor epoxide from March to December 2004 were <0.035 – 1.30 ng/L (Table 4-7). The highest concentration of heptachlor epoxide was used in October (1.30 ± 2.25 ng/L), while the lowest concentration was in March, April, May, June, July, and December (<0.035 ng/L) (Figure 4-13).

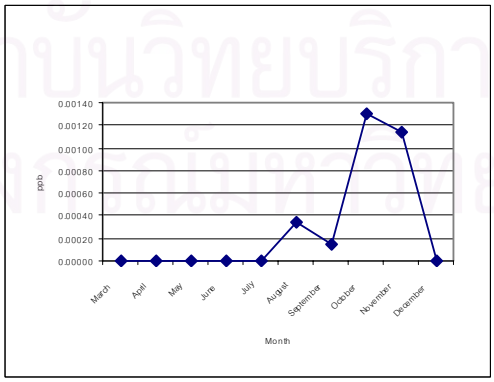


Figure 4-13 Concentrations of heptachlor epoxide in Klong 7 water during March to December 2004

Endosulfan I

The range concentrations of endosulfan I from March to December 2004 were 1.36 – 15.26 ng/L (Table 4-7). The highest concentration of endosulfan I was used in August (15.26 ± 10.78 ng/L), while the lowest concentration was in May (1.36 ± 1.18 ng/L) (Figure 4-14).

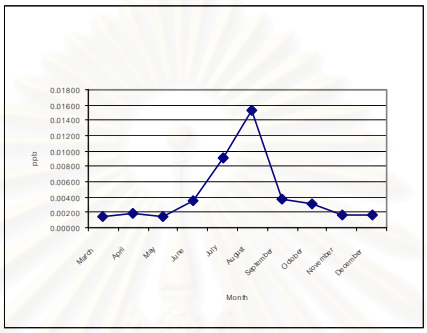


Figure 4-14 Concentrations of endosulfan I in Klong 7 water during March to December 2004

4, 4'-DDE

The range concentrations of 4, 4'-DDE from March to December 2004 were <0.04 – 2.80 ng/L (Table 4-7). The highest concentration of 4, 4'-DDE was used in April (2.80 ± 1.47 ng/L), while the lowest concentration was in March, May, August, September, October, and November (<0.04 ng/L) (Figure 4-15).

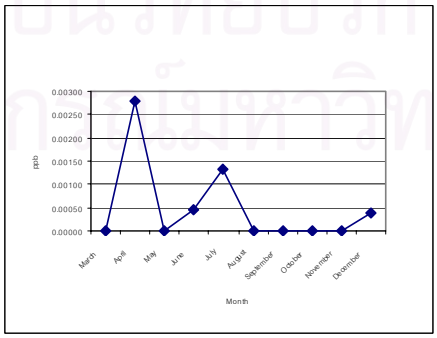


Figure 4-15 Concentrations of 4, 4'-DDE in Klong 7 water during March to December 2004

Dieldrin

The range concentrations of dieldrin from March to December 2004 were 0.87 – 5.81 ng/L (Table 4-7). The highest concentration of dieldrin was used in March (5.81 ± 0.86 ng/L), while the lowest concentration was in October (0.87 ± 1.14 ng/L) (Figure 4-16).

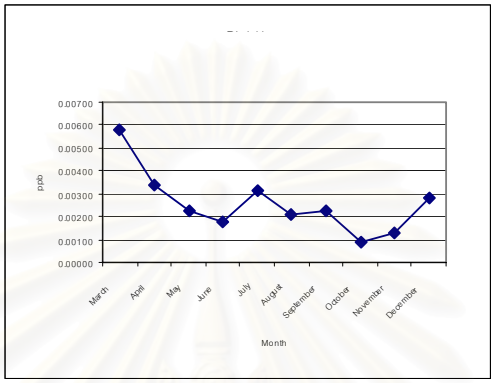


Figure 4-16 Concentrations of dieldrin in Klong 7 water during March to December 2004

Endrin

The range concentrations of endrin from March to December 2004 were <0.10 – 8.69 ng/L (Table 4-7). The highest concentration of endrin was used in July (8.69 ± 7.11 ng/L), while the lowest concentration was in April and May (<0.10 ng/L) (Figure 4-17).

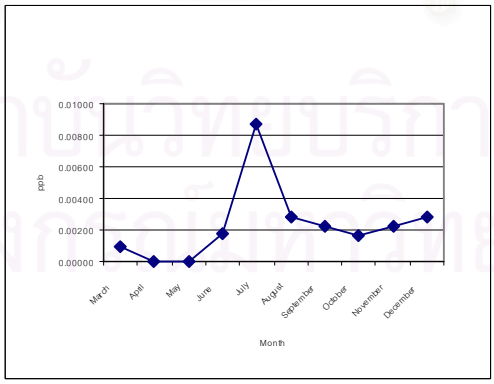


Figure 4-17 Concentrations of endrin in Klong 7 water during March to December 2004

4, 4'-DDD

The range concentrations of 4, 4'-DDD from March to December 2004 were <0.09 – 1.93 ng/L (Table 4-7). The highest concentration of 4, 4'-DDD was used in September (1.93 ± 2.59 ng/L), while the lowest concentration was in March, April, May, June, July, and August (<0.09 ng/L) (Figure 4-18).

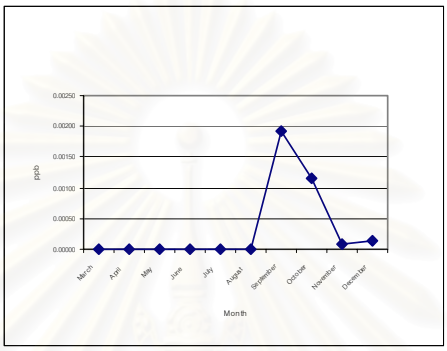


Figure 4-18 Concentrations of 4, 4'-DDD in Klong 7 water during March to December 2004

Endosulfan II

The range concentrations of endosulfan II from March to December 2004 were 0.65 – 14.01 ng/L (Table 4-7). The highest concentration of endosulfan II was used in August (14.01 ± 10.92 ng/L), while the lowest concentration was in May (0.65 ± 0.56 ng/L) (Figure 4-19).

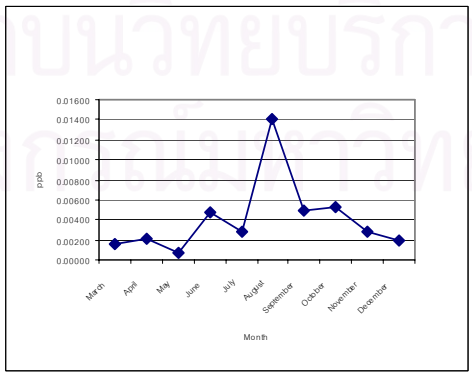


Figure 4-19 Concentrations of endosulfan II in Klong 7 water during March to December 2004

4, 4'-DDT

The range concentrations of 4, 4'-DDT from March to December 2004 were 11.51 – 22.84 ng/L (Table 4-7). The highest concentration of 4, 4'-DDT was used in July (22.84 ± 2.34 ng/L), while the lowest concentration was in May (11.51 ± 0.95 ng/L) (Figure 4-20).

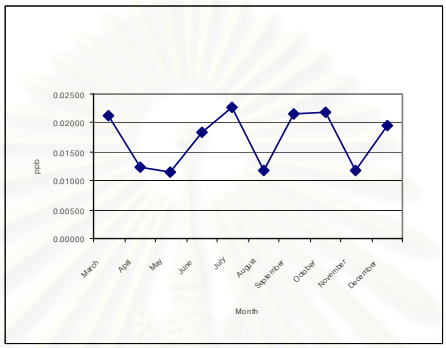


Figure 4-20 Concentrations of 4, 4'-DDT in Klong 7 water during March to December 2004

Endrin aldehyde

The range concentrations of endrin aldehyde from March to December 2004 were <0.08 – 2.36 ng/L (Table 4-7). The highest concentration of endrin aldehyde was used in November (2.36 ± 2.99 ng/L), while the lowest concentration was in March, April, June, July, August, and September (<0.08 ng/L) (Figure 4-21).

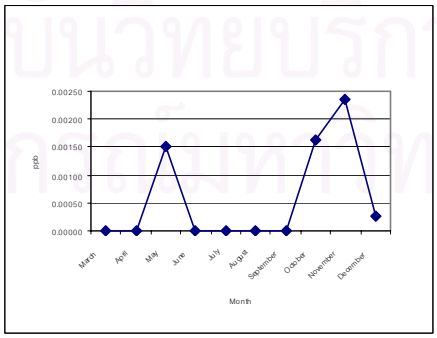


Figure 4-21 Concentrations of endrin aldehyde in Klong 7 water during March to December 2004

Endosulfan sulfate

The range concentrations of endosulfan sulfate from March to December 2004 were 14.11 – 427.80 ng/L (Table 4-7). The highest concentration of endosulfan sulfate was used in August (427.8 $\pm$ 79.63 ng/L), while the lowest concentration was in October (14.11 $\pm$ 6.00 ng/L) (Figure 4-22).

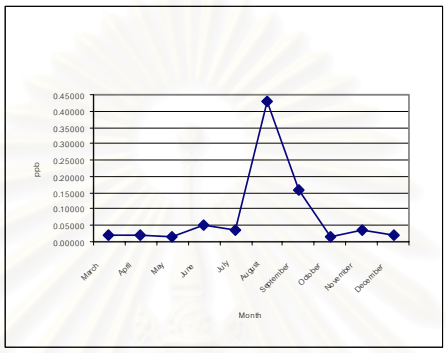


Figure 4-22 Concentrations of endosulfan sulfate in Klong 7 water during March to December 2004

Methoxychlor

The range concentrations of methoxychlor from March to December 2004 were <0.167 – 4.32 ng/L (Table 4-7). The highest concentration of methoxychlor was used in April (4.32 $\pm$ 3.74 ng/L), while low concentration was in March, May, June, July, October, and November (<0.167 ng/L) (Figure 4-23).

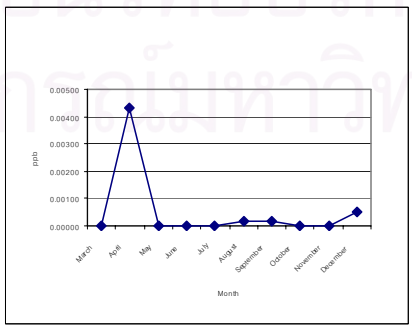


Figure 4-23 Concentrations of methoxychlor in Klong 7 water during March to December 2004

The maximum concentrations of 17 organochlorine pesticides were endosulfan sulfate = 427.80 ng/L, 4, 4'-DDT = 22.84 ng/L, endosulfan I = 15.26 ng/L, endosulfan II = 14.01 ng/L, γ BHC = 8.74 ng/L, endrin = 8.69 ng/L, dieldrin = 5.81 ng/L, aldrin = 5.55 ng/L, δ BHC = 4.62 ng/L, methoxychlor = 4.32 ng/L, α BHC = 3.75 ng/L, heptachlor and β BHC = 2.96 ng/L, 4, 4'- DDE = 2.80 ng/L, endrin aldehyde = 2.50 ng/L, 4, 4'- DDD = 1.93 ng/L, and heptachlor = 1.30 ng/L. The results indicate that many types of pesticide residues still exist as relevant pollutants. It is important to review other previous works related with the contamination of organochlorine pesticides in North of Thailand rivers, because the Klong 7 can accumulate a certain amount of pesticides from pollution sources, north of Thailand. Disyawongs (1979) studied the Lower Pasak river found organochlorine pesticide residues in water such as heptachlor = 0.03 ng/L, heptachlor epoxide = 0.77 ng/L, 4, 4'-DDE = 0.19 ng/L, and α BHC = 0.11 ng/L. On the other hand, Tayapatch et al. (1994) reported that the quantitative analysis of organochlorine pesticide residues in water at Nakhon Sawan, Sakon Nakhon, and Phayao in 1989 found 5 kinds of pesticides; lindane, heptachlor, aldrin, dieldrin, and DDT and DDT derivatives, which were about 0.01-0.12 ppb. Therefore, the organochlorine pesticide residues at Klong 7 may be the combination of pesticides from local area and other areas, north of Rangsit agricultural area.

In the summer (March and April), the most frequent compounds found were γ BHC, dieldrin, methoxychlor, and 4, 4'-DDE, respectively. In the rainy season (May to October), the most frequent compounds found were endosulfan sulfate, endosulfan I, endosulfan II, endrin, aldrin, δ BHC, heptachlor = β BHC, 4, 4'-DDD, and heptachlor epoxide, respectively. Organochlorine pesticide residues found in both summer and rainy season were 4, 4'-DDT, α BHC, and endrin adehyde. The result demonstrates that most who usage of organochlorine pesticides were in rainy season because most agriculture activities begin in the rainy season.

Moreover, the results of this study demonstrate that 17 organochlorine pesticides were found at Klong 7, Pathum Thani Province. However, the residues did not exceed the Maximum Allowable Concentration in drinking water standards (Chulin, 2002).

Relation of the residues in Klong 7 Pathum Thani Province to histological change of Siamese glassfish *Parambassis siamensis*

Water in Klong 7 contained many types of pesticide residues. The chemical interaction between them may enhance their toxicities when two substances have the same physiological function. Their effects may be simply additional (synergist) (Stanley, 2003). This experiment demonstrated that endosulfan, one of many pesticides found in Klong 7, can cause the histopathological change of fish liver. Therefore, the pesticide residues in Klong 7 should affect other aquatic organisms as well. Heath Implications of Environmental Pollutants (2003) reported that DDT, DDT metabolites, chlordane, and endosulfan can cause liver in long term exposures to low levels.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. The histopathological investigation showed the cellular injury which including lipid droplet and hyaline granule accumulation, sinusoid dilation and blood congestion, detachment of endothelial layer of blood vessel, infiltration of macrophage, and necrosis on the fish liver in response to Klong 7 water in summer, Klong 7 water in rainy season, and endosulfan treated fish.

2. Histochemical study showed that the accumulation of both lipid droplets and glycogen deposits were changed on the fish liver in response to Klong 7 water in summer, Klong 7 water in rainy season, and endosulfan treated fish.

3. The relative liver weight index of summer group in Klong 7 was higher than rainy season group in Klong 7, endosulfan treated group, and normal value.

4. Seventeen organochlorine pesticide residues were found in the water at Klong 7, Pathum Thani Province i.e. α BHC, γ BHC, β BHC, heptachlor, δ BHC, aldrin, heptachlor epoxide, endosulfan I, 4, 4'-DDE, dieldrin, endrin, 4, 4'-DDD, endosulfan II, 4, 4'-DDT, endrin aldehyde, endosulfan sulfate, and methoxychlor. However, the residues did not exceed the Maximum Allowable Concentration in drinking water standards.

5. Most organochlorine pesticide residues were higher in rainy season than in summer may indicate the higher use of organochlorine pesticides in paddy fields and other farm lands during rainy season.

6. The laboratory experiment on the effect of endosulfan on the Siamese glassfish *Parambassis siamensis* showed that the contaminated water at Klong 7, Pathum Thani Province in capable of inducing both histopathological and histochemical alterations.

RECOMMENDATIONS

1. The laboratory study on subchronic toxicity should be performed for other organochlorine pesticides found in field study.

2. The laboratory study on subchronic toxicity should treat two organochlorine pesticides for synergistic effect.

3. The effect of physiological parameter occurred in the fish liver such as SGOT and SGPT should be studied.

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Appendices

Appendix A

Chemical reagents and instruments

Histological study

For culturing and testing of potential toxicity

Apparatuses and equipments

1. 14 L glass chamber
2. 300 L aquarium tank
3. Carbon filter chamber
4. Air pump, air line, and air stones
5. pH and DO meters
6. Thermometer
7. Venire caliper
8. Electronic balances
9. Beakers, Volumetric flasks, graduated cylinders, pipettes, droppers, and other glassware

Chemical agent

1. Ethanol 95%
2. 10% formalin in Phosphate buffer

For tissue study processing

Apparatuses and equipments

1. Hot air oven
2. Beakers, Volumetric flasks, graduated cylinders, pipettes, droppers, and other glassware
3. Rotary Microtome
4. Cryostat Rotary microtome
5. pH-meter
6. Light Microscope

Chemical agent

1. Ethanol 95% for preparing the series grade of ethanol
2. Paraplast
3. N-Butyl

Chemical agent (Conc.)

4. Xylene
5. Tissuetek
6. 1% Alcoholic Eosin
7. Glycerol anhydrous
8. Mayer's haematoxylin
9. Permount
10. Periodic acid-schiff (PAS) staining system
11. Oil red O
12. Acetone

Water organochlorine pesticide residues study

Sampling equipment

1. 1L Grab sample bottle
2. pH meter
3. DO meter
4. Thermometer
5. Ice buck

Apparatuses and equipments

1. Separatory funnel
2. Chromatographic column
3. Turbovap tube
4. Ground bottom
5. Snyder column, Kuder/Danish
6. Vials-2, 5, 10, and 15 mL
7. Turbovap® II
8. Analytical balance
9. Gas chromatograph
10. N_2, H_2, and He_2

Reagents

1. Standard mixed organochlorine pesticides
2. Distilled water
3. Dichloromethane
4. Hexane
5. Sodium sulphate anhydrous
6. glass wool
7. Florisil
8. Petroleum ether
9. 6% diethyl ether in petroleum ether
10. 15% diethyl ether in petroleum ether

Appendix B

Fish data and water data

Fish data

Data sheet of control group (n=30)

Number	Body length (cm)	Body weight (g)	Liver weight (g)	% relative liver weight
1	4.50	2.21	0.02	0.90
2	4.20	2.02	0.01	0.50
3	4.20	1.70	0.02	1.18
4	4.30	2.12	0.05	2.36
5	4.20	2.06	0.02	0.97
6	3.80	1.62	0.01	0.62
7	4.10	1.74	0.01	0.57
8	4.00	1.44	0.01	0.69
9	4.10	1.38	0.02	1.45
10	3.80	1.55	0.01	0.65
11	4.00	1.49	0.01	0.67
12	3.40	1.58	0.03	1.90
13	3.40	1.66	0.05	3.01
14	3.20	1.30	0.02	1.54
15	3.50	1.38	0.02	1.45
16	3.50	1.39	0.03	2.16
17	3.20	1.02	0.01	0.98
18	3.40	1.60	0.04	2.50
19	4.20	2.14	0.03	1.47
20	3.00	0.90	0.01	1.11
Mean	3.8000	1.6100	0.0215	1.3340
SE	0.0981	0.0787	0.0029	0.1618

Data sheet of summer group (n=60)

Number	Body length (cm)	Body weight (g)	Liver weight (g)	% relative liver weight
1	6.20	5.85	0.10	1.71
2	4.20	2.64	0.04	1.52
3	4.60	3.62	0.05	1.38
4	4.00	2.85	0.04	1.40
5	4.80	3.49	0.07	2.01
6	4.10	2.14	0.05	2.34
7	3.80	1.74	0.05	2.87
8	4.00	2.38	0.05	2.10
9	4.10	2.40	0.05	2.08
10	4.30	2.53	0.05	1.98
11	4.10	2.33	0.04	1.72
12	3.80	1.65	0.01	0.61
13	6.20	6.14	0.08	1.30
14	4.30	2.47	0.05	2.02
15	4.30	2.52	0.07	2.78
16	4.00	2.26	0.07	3.10
17	4.20	2.39	0.10	4.18
18	4.60	3.18	0.06	1.89
19	4.20	2.49	0.05	2.01
20	3.90	2.00	0.05	2.50
21	4.00	2.17	0.03	1.38
22	4.60	2.91	0.07	2.41
23	4.10	2.37	0.06	2.53
24	4.10	2.20	0.05	2.27
25	4.00	2.31	0.06	2.60
26	4.40	2.69	0.06	2.23
27	4.30	3.00	0.08	2.67
28	4.70	3.31	0.09	2.72
29	4.80	3.68	0.09	2.45
30	4.50	2.95	0.06	2.03

Data sheet of summer group (n=60) (Conc.)

Number	Body length (cm)	Body weight (g)	Liver weight (g)	% relative liver weight
31	4.40	3.22	0.04	1.24
32	4.40	3.08	0.06	1.95
33	4.20	2.70	0.03	1.11
34	4.30	2.67	0.04	1.50
35	4.00	2.55	0.05	1.96
36	4.40	2.75	0.08	2.91
37	4.60	3.40	0.04	1.17
38	4.20	2.68	0.07	2.61
39	4.60	2.91	0.05	1.72
40	4.70	3.11	0.11	2.06
41	4.30	2.85	0.11	3.86
42	4.10	2.34	0.07	2.99
43	4.00	2.31	0.05	2.16
44	4.50	3.00	0.07	2.33
45	4.40	3.04	0.09	2.96
46	4.30	2.53	0.06	2.37
47	4.50	2.90	0.10	3.45
48	4.40	2.59	0.06	2.32
49	4.20	2.72	0.06	2.17
50	4.50	3.10	0.09	2.90
51	4.20	2.72	0.10	3.68
52	4.20	2.61	0.09	3.45
53	4.40	2.88	0.08	2.77
54	4.30	2.56	0.07	2.73
55	4.10	2.66	0.06	2.26
56	4.30	2.87	0.08	2.78
57	4.60	3.35	0.09	3.83
58	4.30	2.75	0.06	2.16
59	4.00	2.38	0.05	2.10
60	4.00	2.34	0.05	2.14
Mean	4.3400	2.8000	0.0640	2.3190
SE	0.0500	0.0900	0.0027	0.0940

Data sheet of rainy season group (n=30)

Number	Body length (cm)	Body weight (g)	Liver weight (g)	% relative liver weight
1	4.30	2.72	0.03	1.10
2	4.40	235.00	0.02	0.85
3	3.60	1.63	0.01	0.61
4	4.30	2.45	0.04	1.63
5	4.50	2.59	0.04	1.54
6	4.50	2.54	0.05	1.97
7	4.30	2.11	0.05	2.37
8	4.50	2.40	0.03	1.25
9	4.10	1.84	0.02	1.09
10	4.30	2.25	0.03	1.33
11	4.30	2.31	0.03	1.30
12	4.00	1.72	0.01	0.58
13	4.40	2.49	0.03	1.20
14	3.90	1.53	0.03	1.96
15	4.10	1.43	0.01	0.70
16	3.80	1.57	0.02	1.27
17	3.50	1.41	0.01	0.71
18	3.60	1.46	0.01	0.68
19	3.60	1.29	0.03	2.33
20	4.00	1.89	0.03	1.59
21	3.80	1.53	0.02	1.31
22	3.90	1.89	0.10	5.29
23	3.70	1.51	0.01	0.66
24	3.80	1.30	0.01	0.80
25	3.30	1.63	0.02	1.23
26	3.50	1.11	0.01	0.90
27	3.30	1.14	0.01	0.88
28	3.20	1.05	0.01	0.95
29	4.10	1.76	0.01	0.57
30	4.20	2.02	0.02	0.99
Mean	3.9600	1.8300	0.0220	1.3210
SE	0.0700	0.0900	0.0022	0.1640

Data sheet of treated endosulfan 0.01 μg/L group (n=10)

Number	Body length (cm)	Body weight (g)	Liver weight (g)	% relative liver weight
1	3.30	1.02	0.01	0.98
2	3.10	0.95	0.01	1.05
3	3.40	1.28	0.02	1.56
4	3.30	1.12	0.01	0.89
5	3.00	0.89	0.01	1.12
6	3.60	1.51	0.03	1.98
7	3.20	1.06	0.02	1.88
8	3.80	1.57	0.03	1.91
9	3.20	0.92	0.01	1.09
10	3.60	1.24	0.01	0.80
Mean	3.3500	1.1560	0.0160	1.3260
SE	0.7923	0.0758	0.0027	0.1450

Data sheet of treated endosulfan 0.06 μg/L group (n=10)

Number	Body length (cm)	Body weight (g)	Liver weight (g)	% relative liver weight
1	3.70	1.25	0.01	0.80
2	3.20	1.06	0.02	1.88
3	3.20	0.93	0.01	1.07
4	3.60	1.39	0.01	0.72
5	3.80	1.59	0.02	1.26
6	3.30	0.98	0.01	1.02
7	3.00	0.87	0.01	1.15
8	3.10	0.90	0.01	1.11
9	3.60	1.58	0.03	1.90
10	3.50	1.39	0.02	1.44
Mean	3.4000	1.1940	0.0150	1.2350
SE	0.0869	0.0886	0.0022	0.1270

Data sheet of treated endosulfan 0.5 μg/L group (n=10)

Number	Body length (cm)	Body weight (g)	Liver weight (g)	% relative liver weight
1	3.40	1.12	0.01	0.89
2	3.40	1.25	0.02	1.60
3	3.40	1.08	0.01	0.92
4	3.50	1.33	0.02	1.50
5	3.50	1.14	0.01	0.87
6	3.10	0.87	0.01	1.15
7	3.80	1.62	0.03	1.85
8	3.20	1.26	0.02	1.59
9	3.60	1.40	0.03	2.14
10	3.80	1.60	0.05	3.13
Mean	3.4700	1.2670	0.0210	1.5640
SE	0.0716	0.0736	0.0041	0.2210

Data sheet of treated endosulfan 1 μg/L group (n=10)

Number	Body length (cm)	Body weight (g)	Liver weight (g)	% relative liver weight
1	3.20	1.11	0.01	0.90
2	3.40	1.16	0.02	1.72
3	3.30	1.17	0.01	0.85
4	3.40	1.19	0.02	1.68
5	4.20	1.71	0.03	1.75
6	4.20	2.10	0.03	1.43
7	4.30	2.14	0.04	1.87
8	3.80	1.70	0.02	1.17
9	3.40	1.67	0.05	3.00
10	3.60	1.69	0.05	2.96
Mean	3.6800	1.5640	0.0280	1.7330
SE	0.1315	0.1225	0.0047	0.2361

Water quality during a positive group and control group toxicity test.

Parameter	Range
Temperature of water (°C)	26-28
Temperature of air (°C)	30-31
Dissolved oxygen (mg/L)	5.4-5.8
pH	7.0-7.4

Water data

Organochlorine pesticide residues at Klong 7 Pathum Thani Province at March to December 2004 (μg/L)

March site A, B, and C

March, site A	Replicated 1	Replicated 2	Replicated 3	Mean	SD	%RSD
a BHC	0.00000	0.00000	0.00383	0.00128	0.00221	173
g BHC	0.00519	0.00600	0.00470	0.00530	0.00066	12
b BHC	0.00000	0.00000	0.00349	0.00116	0.00201	173
Heptachlor	0.00604	0.00671	0.00000	0.00425	0.00369	87
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00000	0.00374	0.00000	0.00125	0.00216	173
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00286	0.00294	0.00258	0.00279	0.00019	7
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00286	0.00588	0.00596	0.00490	0.00177	36
Endril	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDT	0.02222	0.01826	0.02619	0.02222	0.00397	18
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.00928	0.01095	0.01138	0.01054	0.00111	11
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00000	0.00413	0.00000	0.00138	0.00238	173
g BHC	0.00497	0.00522	0.00552	0.00524	0.00028	5
b BHC	0.00000	0.00641	0.00000	0.00214	0.00370	173
Heptachlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00405	0.00502	0.00388	0.00432	0.00062	14
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00669	0.00425	0.00685	0.00593	0.00146	25
Endril	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00000	0.00287	0.00000	0.00096	0.00166	173
4-4' DDT	0.02130	0.02100	0.02064	0.02098	0.00033	2
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.01610	0.01632	0.01672	0.01638	0.00031	2
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00000	0.00444	0.00394	0.00279	0.00243	87
g BHC	0.00722	0.00620	0.00833	0.00725	0.00107	15
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00403	0.00413	0.00376	0.00397	0.00019	5
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00286	0.00261	0.00000	0.00182	0.00158	87
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00519	0.00686	0.00777	0.00661	0.00131	20
Endril	0.00422	0.00470	0.00000	0.00297	0.00259	87
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00318	0.00394	0.00497	0.00403	0.00090	22
4-4' DDT	0.02255	0.01803	0.02040	0.02033	0.00226	11
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.02750	0.03192	0.02839	0.02927	0.00234	8
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0

April site A, B, and C

April, site A	Replicated 1	Replicated 2	Replicated 3	Mean	SD	%RSD
a BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
g BHC	0.00541	0.04090	0.00479	0.01703	0.02067	121
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.07751	0.00000	0.00000	0.02584	0.04475	173
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00352	0.00357	0.00361	0.00357	0.00005	1
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00339	0.00000	0.00000	0.00113	0.00196	173
4-4' DDE	0.00000	0.00462	0.00433	0.00298	0.00259	87
Dieldrin	0.00430	0.00451	0.00482	0.00454	0.00026	6
Endril	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDT	0.01700	0.00955	0.01140	0.01265	0.00388	31
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.00804	0.00811	0.00806	0.00807	0.00004	1
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
g BHC	0.00421	0.00431	0.00464	0.00439	0.00023	5
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00428	0.00443	0.00417	0.00429	0.00013	3
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00356	0.00358	0.00328	0.00347	0.00017	5
4-4' DDE	0.00392	0.00421	0.00436	0.00416	0.00022	5
Dieldrin	0.00000	0.00310	0.00452	0.00254	0.00231	91
Endril	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00394	0.00410	0.00390	0.00398	0.00011	3
4-4' DDT	0.01150	0.01114	0.01534	0.01266	0.00233	18
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.03068	0.02177	0.02135	0.02460	0.00527	21
Methoxychlor	0.00627	0.00646	0.00674	0.00649	0.00024	4
a BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
g BHC	0.00499	0.00431	0.00511	0.00480	0.00043	9
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.00000	0.00578	0.00193	0.00334	173
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00341	0.00333	0.00334	0.00336	0.00005	1
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00000	0.00000	0.00317	0.00106	0.00183	173
4-4' DDE	0.00375	0.00000	0.00000	0.00125	0.00217	173
Dieldrin	0.00000	0.00454	0.00443	0.00299	0.00259	87
Endril	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00000	0.00360	0.00293	0.00218	0.00191	88
4-4' DDT	0.01068	0.01233	0.01217	0.01173	0.00091	8
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.02537	0.02366	0.02190	0.02364	0.00174	7
Methoxychlor	0.00627	0.00645	0.00670	0.00647	0.00022	3

May site A, B, and C

May, site A	Replicated 1	Replicated 2	Replicated 3	Mean	SD	%RSD
a BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
g BHC	0.00473	0.00440	0.00608	0.00507	0.00089	18
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00341	0.00350	0.03498	0.01396	0.01820	130
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00374	0.00456	0.00294	0.00375	0.00081	22
Endril	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDT	0.01375	0.01187	0.01219	0.01260	0.00101	8
Endril aldehyde	0.00383	0.00000	0.00383	0.00255	0.00221	87
Endosulfan sulfate	0.00839	0.00915	0.00905	0.00886	0.00041	5
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00414	0.00384	0.00386	0.00395	0.00017	4
g BHC	0.00493	0.00459	0.00509	0.00487	0.00026	5
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.00000	0.06852	0.02284	0.03956	173
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00405	0.00000	0.00000	0.00135	0.00234	173
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00290	0.00000	0.00321	0.00204	0.00177	87
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00464	0.00000	0.00000	0.00155	0.00268	173
Endril	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00292	0.00000	0.00000	0.00097	0.00169	173
4-4' DDT	0.01586	0.00136	0.01567	0.01096	0.00832	76
Endril aldehyde	0.00300	0.00000	0.00000	0.00100	0.00173	173
Endosulfan sulfate	0.01923	0.01756	0.01828	0.01836	0.00084	5
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00414	0.00384	0.00386	0.00395	0.00017	4
g BHC	0.00493	0.00459	0.00509	0.00487	0.00026	5
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.00000	0.06852	0.02284	0.03956	173
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00405	0.00000	0.00000	0.00135	0.00234	173
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00290	0.00000	0.00321	0.00204	0.00177	87
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00464	0.00000	0.00000	0.00155	0.00268	173
Endril	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00292	0.00000	0.00000	0.00097	0.00169	173
4-4' DDT	0.01586	0.00136	0.01567	0.01096	0.00832	76
Endril aldehyde	0.00300	0.00000	0.00000	0.00100	0.00173	173
Endosulfan sulfate	0.01923	0.01756	0.01828	0.01836	0.00084	5
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0

June site A, B, and C

June, site A	Replicated 1	Replicated 2	Replicated 3	Mean	SD	%RSD
a BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
g BHC	0.00830	0.00663	0.00541	0.00678	0.00145	21
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.00707	0.00000	0.00236	0.00408	173
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00298	0.00000	0.00000	0.00099	0.00172	173
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00260	0.00000	0.00251	0.00170	0.00148	87
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00477	0.00442	0.00349	0.00423	0.00066	16
Endril	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDT	0.01584	0.01660	0.01451	0.01565	0.00106	7
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.06075	0.06046	0.06183	0.06101	0.00072	1
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
g BHC	0.00475	0.00456	0.00775	0.00569	0.00179	31
b BHC	0.00000	0.00000	0.00363	0.00121	0.00210	173
Heptachlor	0.00000	0.00000	0.00705	0.00235	0.00407	173
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00326	0.00329	0.00344	0.00333	0.00010	3
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00334	0.00346	0.00350	0.00343	0.00008	2
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endril	0.00438	0.00000	0.00000	0.00146	0.00253	173
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.02763	0.00297	0.00000	0.01020	0.01517	149
4-4' DDT	0.01751	0.01651	0.01926	0.01776	0.00139	8
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.05353	0.05852	0.06261	0.05822	0.00455	8
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00386	0.00381	0.00432	0.00400	0.00028	7
g BHC	0.00505	0.00884	0.00389	0.00593	0.00259	44
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.01016	0.00727	0.00662	0.00802	0.00188	24
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00335	0.00326	0.00319	0.00327	0.00008	2
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00416	0.00583	0.00577	0.00525	0.00095	18
4-4' DDE	0.00000	0.00398	0.00000	0.00133	0.00230	173
Dieldrin	0.00000	0.00299	0.00000	0.00100	0.00173	173
Endril	0.00379	0.00379	0.00393	0.00384	0.00008	2
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00360	0.00452	0.00383	0.00398	0.00048	12
4-4' DDT	0.02783	0.01733	0.02106	0.02207	0.00532	24
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.02915	0.02990	0.02857	0.02921	0.00067	2
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0

July site A, B, and C

July, site A	Replicated 1	Replicated 2	Replicated 3	Mean	SD	%RSD
a BHC	0.00446	0.00385	0.00438	0.00423	0.00033	8
g BHC	0.00522	0.00948	0.00397	0.00622	0.00289	46
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.01190	0.00772	0.00687	0.00883	0.00269	30
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00341	0.00328	0.00331	0.00333	0.00007	2
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00420	0.00595	0.00593	0.00536	0.00100	19
4-4' DDE	0.00405	0.00393	0.00394	0.00397	0.00007	2
Dieldrin	0.00000	0.00319	0.00000	0.00106	0.00184	173
Endril	0.04272	0.00390	0.00406	0.01689	0.02237	132
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00376	0.00461	0.00395	0.00411	0.00045	11
4-4' DDT	0.03494	0.01778	0.01830	0.02367	0.00976	41
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.03102	0.03156	0.03031	0.03096	0.00063	2
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00442	0.00390	0.00437	0.00423	0.00029	7
g BHC	0.00535	0.00492	0.00472	0.00500	0.00032	6
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.01203	0.00742	0.00648	0.00607	94
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00347	0.00354	0.00340	0.00347	0.00007	2
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00401	0.00352	0.00304	0.00352	0.00049	14
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00381	0.00305	0.00349	0.00345	0.00038	11
Endril	0.00442	0.00410	0.00485	0.00446	0.00038	8
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00000	0.00280	0.00000	0.00093	0.00162	173
4-4' DDT	0.01788	0.03537	0.02067	0.02464	0.00940	38
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.03323	0.03041	0.03124	0.03163	0.00145	5
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00410	0.00425	0.00000	0.00278	0.00241	87
g BHC	0.00542	0.00605	0.00491	0.00546	0.00057	10
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.01036	0.00589	0.00977	0.00867	0.00243	28
d BHC	0.00511	0.00000	0.00000	0.00170	0.00295	173
Aldrin	0.00356	0.00385	0.00370	0.00370	0.00015	4
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00755	0.04300	0.00405	0.01820	0.02155	118
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00476	0.00497	0.00467	0.00480	0.00015	3
Endril	0.00491	0.00430	0.00492	0.00471	0.00036	8
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00526	0.00000	0.00459	0.00328	0.00286	87
4-4' DDT	0.02009	0.02050	0.02000	0.02020	0.00027	1
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.03792	0.04500	0.03900	0.04064	0.00381	9
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0

August site A, B, and C

August, site A	Replicated 1	Replicated 2	Replicated 3	Mean	SD	%RSD
a BHC	0.00000	0.00178	0.00000	0.00059	0.00103	173
g BHC	0.00368	0.01483	0.00318	0.00723	0.00659	91
b BHC	0.00441	0.00439	0.00309	0.00396	0.00076	19
Heptachlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
d BHC	0.00000	0.00354	0.00000	0.00118	0.00204	173
Aldrin	0.00000	0.00125	0.00000	0.00042	0.00072	173
Heptachlor epoxide	0.00000	0.00000	0.00133	0.00044	0.00077	173
Endosulfan I	0.00306	0.00319	0.00320	0.00315	0.00008	2
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00249	0.00252	0.00253	0.00251	0.00002	1
Endril	0.00152	0.00222	0.00319	0.00231	0.00084	36
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00337	0.00321	0.00338	0.00332	0.00010	3
4-4' DDT	0.01135	0.01099	0.01218	0.01151	0.00061	5
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.45481	0.53187	0.56609	0.51759	0.05700	11
Methoxychlor	0.00131	0.00000	0.00000	0.00044	0.00076	173
a BHC	0.00000	0.00171	0.00000	0.00057	0.00099	173
g BHC	0.00266	0.00000	0.00419	0.00228	0.00212	93
b BHC	0.00335	0.00000	0.00461	0.00265	0.00238	90
Heptachlor	0.00000	0.00186	0.00000	0.00062	0.00107	173
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00000	0.00000	0.00126	0.00042	0.00073	173
Heptachlor epoxide	0.00179	0.00000	0.00000	0.00060	0.00103	173
Endosulfan I	0.00651	0.00275	0.06216	0.02381	0.03327	140
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00278	0.00127	0.00255	0.00220	0.00081	37
Endril	0.00236	0.00298	0.00343	0.00292	0.00054	18
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00553	0.00302	0.06691	0.02515	0.03618	144
4-4' DDT	0.01554	0.01057	0.01110	0.01240	0.00273	22
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.29138	0.23258	0.57329	0.36575	0.18212	50
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00191	0.00161	0.00190	0.00181	0.00017	9
g BHC	0.00000	0.00177	0.00000	0.00059	0.00102	173
b BHC	0.00169	0.00372	0.00140	0.00227	0.00126	56
Heptachlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
d BHC	0.00000	0.00271	0.00000	0.00090	0.00156	173
Aldrin	0.00127	0.00000	0.00120	0.00082	0.00071	87
Heptachlor epoxide	0.00000	0.00169	0.00000	0.00000	0.00000	0
Endosulfan I	0.00308	0.05108	0.00233	0.01883	0.02793	148
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00195	0.00140	0.00113	0.00149	0.00042	28
Endril	0.00288	0.00384	0.00265	0.00312	0.00063	20
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00196	0.03727	0.00145	0.01356	0.02054	151
4-4' DDT	0.01184	0.01189	0.01040	0.01138	0.00085	7
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.29066	0.61870	0.29080	0.40005	0.18935	47
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0

September site A, B, and C

September, site A	Replicated 1	Replicated 2	Replicated 3	Mean	SD	%RSD
a BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
g BHC	0.00328	0.00275	0.00620	0.00408	0.00186	46
b BHC	0.00170	0.00133	0.00163	0.00155	0.00020	13
Heptachlor	0.00303	0.00398	0.00382	0.00361	0.00051	14
d BHC	0.00000	0.00000	0.00269	0.00090	0.00155	173
Aldrin	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00109	0.00136	0.00129	0.00125	0.00014	11
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00216	0.00223	0.00145	0.00195	0.00043	22
Endril	0.00180	0.00218	0.00184	0.00194	0.00021	11
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00143	0.00321	0.00000	0.00155	0.00161	104
4-4' DDT	0.00725	0.00734	0.00638	0.00699	0.00053	8
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.11897	0.13321	0.15240	0.13486	0.01678	12
Methoxychlor	0.00131	0.00000	0.00000	0.00044	0.00076	173
a BHC	0.00184	0.00164	0.00152	0.00167	0.00016	10
g BHC	0.00169	0.00205	0.00352	0.00242	0.00097	40
b BHC	0.00184	0.00114	0.00105	0.00134	0.00043	32
Heptachlor	0.00550	0.00638	0.00582	0.00590	0.00045	8
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00151	0.00124	0.00152	0.00142	0.00016	11
Heptachlor epoxide	0.00000	0.00131	0.00000	0.00044	0.00076	173
Endosulfan I	0.00420	0.00273	0.00195	0.00296	0.00114	39
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00366	0.00376	0.00268	0.00337	0.00060	18
Endril	0.00317	0.00000	0.00237	0.00185	0.00165	89
4-4' DDD	0.00000	0.00159	0.00115	0.00091	0.00082	90
Endosulfan II	0.00207	0.00263	0.00171	0.00214	0.00046	22
4-4' DDT	0.04782	0.03084	0.02348	0.03405	0.01248	37
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.17667	0.17337	0.15260	0.16755	0.01305	8
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00151	0.00173	0.01670	0.00665	0.00871	131
g BHC	0.00194	0.00000	0.01458	0.00551	0.00792	144
b BHC	0.00144	0.00163	0.00134	0.00147	0.00015	10
Heptachlor	0.00448	0.00441	0.00552	0.00480	0.00062	13
d BHC	0.01339	0.01119	0.01430	0.01296	0.00160	12
Aldrin	0.00104	0.00174	0.00165	0.00148	0.00038	26
Heptachlor epoxide	0.00000	0.00090	0.00082	0.00000	0.00000	0
Endosulfan I	0.00928	0.01059	0.00061	0.00683	0.00542	79
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00151	0.00115	0.00146	0.00137	0.00020	14
Endril	0.00274	0.00283	0.00338	0.00298	0.00035	12
4-4' DDD	0.00185	0.00294	0.00981	0.00487	0.00432	89
Endosulfan II	0.01122	0.01282	0.00981	0.01128	0.00151	13
4-4' DDT	0.02003	0.02304	0.02725	0.02344	0.00363	15
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.14098	0.18054	0.21554	0.17902	0.03730	21
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0

October site A, B, and C

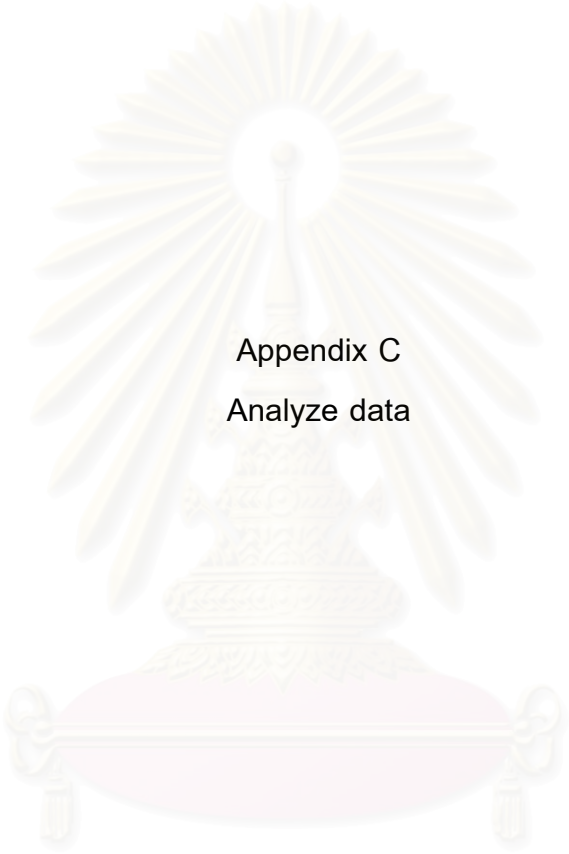
October, site A	Replicated 1	Replicated 2	Replicated 3	Mean	SD	%RSD
a BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
g BHC	0.00600	0.00311	0.00579	0.00497	0.00161	32
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.01936	0.96172	0.01430	0.33179	0.54554	164
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00225	0.00527	0.00257	0.00336	0.00166	49
Heptachlor epoxide	0.00000	0.00318	0.00851	0.00390	0.00430	110
Endosulfan I	0.00220	0.00555	0.00237	0.00337	0.00189	56
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00000	0.00447	0.00201	0.00216	0.00224	104
Endril	0.00000	0.00000	0.00000	0.00000	0.00000	0
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00378	0.01861	0.00611	0.00950	0.00798	84
4-4' DDT	0.02223	0.05160	0.02132	0.03172	0.01723	54
Endril aldehyde	0.00160	0.00595	0.00226	0.00327	0.00234	72
Endosulfan sulfate	0.00673	0.00658	0.00864	0.00732	0.00115	16
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00203	0.00000	0.00218	0.00140	0.00122	87
g BHC	0.00518	0.00236	0.00377	0.00377	0.00141	37
b BHC	0.00133	0.00238	0.00238	0.00203	0.00061	30
Heptachlor	0.00360	0.00824	0.00982	0.00722	0.00323	45
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00205	0.00188	0.00201	0.00198	0.00009	4
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00179	0.00824	0.00155	0.00386	0.00380	98
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00137	0.00000	0.00000	0.00046	0.00079	173
Endril	0.00193	0.00219	0.00237	0.00216	0.00022	10
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00339	0.00883	0.00157	0.00460	0.00378	82
4-4' DDT	0.01619	0.01213	0.01357	0.01396	0.00206	15
Endril aldehyde	0.00155	0.00103	0.00000	0.00086	0.00079	92
Endosulfan sulfate	0.01887	0.01793	0.01925	0.01868	0.00068	4
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00213	0.00210	0.00220	0.00214	0.00005	2
g BHC	0.00360	0.00469	0.00280	0.00370	0.00095	26
b BHC	0.01491	0.00159	0.00122	0.00591	0.00780	132
Heptachlor	0.00727	0.00762	0.00956	0.00815	0.00123	15
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00159	0.00189	0.00216	0.00188	0.00029	15
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00177	0.00191	0.00170	0.00179	0.00011	6
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endril	0.00336	0.00167	0.00365	0.00289	0.00107	37
4-4' DDD	0.00000	0.00000	0.01043	0.00348	0.00602	173
Endosulfan II	0.00116	0.00161	0.00194	0.00157	0.00039	25
4-4' DDT	0.01982	0.01950	0.02022	0.01985	0.00036	2
Endril aldehyde	0.00102	0.00000	0.00131	0.00078	0.00069	89
Endosulfan sulfate	0.01467	0.01714	0.01718	0.01633	0.00144	9
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0

November site A, B, and C

November, site A	Replicated 1	Replicated 2	Replicated 3	Mean	SD	%RSD
a BHC	0.00212	0.00216	0.00214	0.00214	0.00002	1
g BHC	0.00353	0.00306	0.00269	0.00309	0.00042	14
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.01410	0.01329	0.02567	0.01769	0.00693	39
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00205	0.00166	0.00165	0.00179	0.00023	13
Heptachlor epoxide	0.00925	0.00000	0.00000	0.00308	0.00534	173
Endosulfan I	0.00225	0.00223	0.00125	0.00191	0.00057	30
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00173	0.00119	0.00000	0.00097	0.00089	91
Endril	0.00194	0.00191	0.00203	0.00196	0.00006	3
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00513	0.00199	0.00129	0.00280	0.00205	73
4-4' DDT	0.01743	0.01082	0.01094	0.01306	0.00378	29
Endril aldehyde	0.00213	0.00125	0.01405	0.00581	0.00715	123
Endosulfan sulfate	0.03640	0.03532	0.03978	0.03717	0.00233	6
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00201	0.00292	0.00214	0.00236	0.00049	21
g BHC	0.00441	0.00460	0.00567	0.00489	0.00068	14
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.01377	0.01601	0.01891	0.01623	0.00258	16
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00207	0.00162	0.00160	0.00176	0.00027	15
Heptachlor epoxide	0.00103	0.00000	0.00000	0.00034	0.00059	173
Endosulfan I	0.00241	0.00140	0.00154	0.00178	0.00055	31
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00185	0.00000	0.00309	0.00165	0.00156	94
Endril	0.00000	0.00219	0.00227	0.00149	0.00129	87
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00516	0.01033	0.00000	0.00516	0.00517	100
4-4' DDT	0.01923	0.01033	0.01011	0.01322	0.00520	39
Endril aldehyde	0.00225	0.00000	0.00000	0.00075	0.00130	173
Endosulfan sulfate	0.03185	0.03555	0.03721	0.03487	0.00274	8
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00203	0.00221	0.00219	0.00214	0.00010	5
g BHC	0.00361	0.00408	0.00337	0.00369	0.00036	10
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.01134	0.01700	0.01266	0.01367	0.00296	22
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00209	0.00163	0.00162	0.00178	0.00027	15
Heptachlor epoxide	0.00790	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00175	0.00136	0.00115	0.00142	0.00030	21
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00228	0.00000	0.00124	0.00117	0.00114	97
Endril	0.00295	0.00325	0.00375	0.00332	0.00040	12
4-4' DDD	0.00077	0.00000	0.00000	0.00026	0.00044	173
Endosulfan II	0.00000	0.00000	0.00082	0.00027	0.00047	173
4-4' DDT	0.01134	0.01423	0.00187	0.00915	0.00647	71
Endril aldehyde	0.00000	0.00153	0.00000	0.00051	0.00088	173
Endosulfan sulfate	0.03240	0.03073	0.02926	0.03080	0.00157	5
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0

December site A, B, and C

December, site A	Replicated 1	Replicated 2	Replicated 3	Mean	SD	%RSD
a BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
g BHC	0.00444	0.00490	0.00547	0.00494	0.00052	10
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00000	0.00208	0.00439	0.00216	0.00220	102
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00144	0.00113	0.00228	0.00162	0.00060	37
4-4' DDE	0.00000	0.00171	0.00178	0.00116	0.00101	87
Dieldrin	0.00122	0.00362	0.00478	0.00321	0.00182	57
Endril	0.00149	0.00182	0.00288	0.00206	0.00073	35
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00000	0.00000	0.00258	0.00086	0.00149	173
4-4' DDT	0.01547	0.01496	0.01540	0.01528	0.00028	2
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.01395	0.01079	0.01913	0.01462	0.00421	29
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
a BHC	0.00201	0.00000	0.00211	0.00137	0.00119	87
g BHC	0.00333	0.00289	0.00292	0.00305	0.00025	8
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
d BHC	0.00000	0.00232	0.00000	0.00077	0.00134	173
Aldrin	0.01503	0.00142	0.00170	0.00605	0.00778	129
Heptachlor epoxide	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan I	0.00121	0.00132	0.00129	0.00127	0.00006	4
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00185	0.00331	0.00000	0.00172	0.00166	96
Endril	0.00241	0.00245	0.00237	0.00241	0.00004	2
4-4' DDD	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan II	0.00170	0.00101	0.00155	0.00142	0.00036	26
4-4' DDT	0.01465	0.01687	0.01204	0.01452	0.00242	17
Endril aldehyde	0.00000	0.00000	0.00000	0.00000	0.00000	0
Endosulfan sulfate	0.01465	0.01687	0.01204	0.01452	0.00242	17
Methoxychlor	0.00000	0.00000	0.00460	0.00153	0.00266	173
a BHC	0.00245	0.00256	0.00249	0.00250	0.00006	2
g BHC	0.00427	0.00375	0.00414	0.00405	0.00027	7
b BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Heptachlor	0.00000	0.00000	0.00000	0.00000	0.00000	0
d BHC	0.00000	0.00000	0.00000	0.00000	0.00000	0
Aldrin	0.00164	0.00216	0.00192	0.00191	0.00026	14
Heptachlor epoxide	0.00000	0.00120	0.00000	0.00000	0.00000	0
Endosulfan I	0.00164	0.00228	0.00275	0.00222	0.00056	25
4-4' DDE	0.00000	0.00000	0.00000	0.00000	0.00000	0
Dieldrin	0.00177	0.00779	0.00134	0.00363	0.00361	99
Endril	0.00469	0.00360	0.00365	0.00398	0.00062	15
4-4' DDD	0.00000	0.00122	0.00000	0.00041	0.00070	173
Endosulfan II	0.00082	0.00678	0.00224	0.00328	0.00311	95
4-4' DDT	0.02179	0.03662	0.02683	0.02841	0.00754	27
Endril aldehyde	0.00000	0.00224	0.00000	0.00075	0.00129	173
Endosulfan sulfate	0.02924	0.03636	0.03806	0.03455	0.00468	14
Methoxychlor	0.00000	0.00000	0.00000	0.00000	0.00000	0



Appendix C

Analyze data

Oneway

Descriptives

R

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	20	1.3340	.7235	.1618	.9954	1.6726	.50	3.01
dry season	60	2.3193	.7280	9.399E-02	2.1313	2.5074	.61	4.18
wet season	30	1.3213	.8980	.1640	.9860	1.6567	.57	5.29
0.01 ppb endosulfan	10	1.3260	.4584	.1450	.9981	1.6539	.80	1.98
0.06 ppb endosulfan	10	1.2350	.4016	.1270	.9477	1.5223	.72	1.90
0.5 ppb endosulfan	10	1.5640	.6987	.2210	1.0642	2.0638	.87	3.13
1 ppb endosulfan	10	1.7330	.7465	.2361	1.1990	2.2670	.85	3.00
Total	150	1.7604	.8614	7.033E-02	1.6214	1.8994	.50	5.29

Test of Homogeneity of Variances

R

Levene Statistic	df1	df2	Sig.
.537	6	143	.779

ANOVA

R

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	33.205	6	5.534	10.230	.000
Within Groups	77.357	143	.541		
Total	110.562	149			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: R

	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	control	dry season	-.9853*	.1899	.000	-1.3607	-.6099
		wet season	1.267E-02	.2123	.953	-.4070	.4324
		0.01 ppb endosulfan	8.000E-03	.2849	.978	-.5551	.5711
		0.06 ppb endosulfan	9.900E-02	.2849	.729	-.4641	.6621
		0.5 ppb endosulfan	-.2300	.2849	.421	-.7931	.3331
		1 ppb endosulfan	-.3990	.2849	.163	-.9621	.1641
	dry season	control	.9853*	.1899	.000	.6099	1.3607
		wet season	.9980*	.1645	.000	.6729	1.3231
		0.01 ppb endosulfan	.9933*	.2512	.000	.4967	1.4899
		0.06 ppb endosulfan	1.0843*	.2512	.000	.5877	1.5809
		0.5 ppb endosulfan	.7553*	.2512	.003	.2587	1.2519
		1 ppb endosulfan	.5863*	.2512	.021	8.975E-02	1.0829
	wet season	control	-1.2667E-02	.2123	.953	-.4324	.4070
		dry season	-.9980*	.1645	.000	-1.3231	-.6729
		0.01 ppb endosulfan	-4.6667E-03	.2686	.986	-.5355	.5262
		0.06 ppb endosulfan	8.633E-02	.2686	.748	-.4445	.6172
		0.5 ppb endosulfan	-.2427	.2686	.368	-.7735	.2882
		1 ppb endosulfan	-.4117	.2686	.128	-.9425	.1192
	0.01 ppb endosulfan	control	-8.0000E-03	.2849	.978	-.5711	.5551
		dry season	-.9933*	.2512	.000	-1.4899	-.4967
		wet season	4.667E-03	.2686	.986	-.5262	.5355
		0.06 ppb endosulfan	9.100E-02	.3289	.782	-.5592	.7412
		0.5 ppb endosulfan	-.2380	.3289	.471	-.8882	.4122
		1 ppb endosulfan	-.4070	.3289	.218	-1.0572	.2432
	0.06 ppb endosulfan	control	-9.9000E-02	.2849	.729	-.6621	.4641
		dry season	-1.0843*	.2512	.000	-1.5809	-.5877
		wet season	-8.6333E-02	.2686	.748	-.6172	.4445
		0.01 ppb endosulfan	-9.1000E-02	.3289	.782	-.7412	.5592
		0.5 ppb endosulfan	-.3290	.3289	.319	-.9792	.3212
		1 ppb endosulfan	-.4980	.3289	.132	-1.1482	.1522
	0.5 ppb endosulfan	control	.2300	.2849	.421	-.3331	.7931
		dry season	-.7553*	.2512	.003	-1.2519	-.2587
		wet season	.2427	.2686	.368	-.2882	.7735
		0.01 ppb endosulfan	.2380	.3289	.471	-.4122	.8882
		0.06 ppb endosulfan	.3290	.3289	.319	-.3212	.9792
		1 ppb endosulfan	-.1690	.3289	.608	-.8192	.4812
	1 ppb endosulfan	control	.3990	.2849	.163	-.1641	.9621
		dry season	-.5863*	.2512	.021	-1.0829	-8.9748E-02
		wet season	.4117	.2686	.128	-.1192	.9425
		0.01 ppb endosulfan	.4070	.3289	.218	-.2432	1.0572
		0.06 ppb endosulfan	.4980	.3289	.132	-.1522	1.1482
		0.5 ppb endosulfan	.1690	.3289	.608	-.4812	.8192

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

R

	GROUP	N	Subset for alpha = .05	
			1	2
Duncan[a,b]	0.06 ppb endosulfan	10	1.2350	
	wet season	30	1.3213	
	0.01 ppb endosulfan	10	1.3260	
	control	20	1.3340	
	0.5 ppb endosulfan	10	1.5640	
	1 ppb endosulfan	10	1.7330	
	dry season	60		2.3193
	Sig.		.120	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 14.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Note;

wet season = rainy season

dry season = summer

ppb = μg/L

BIOGRAPHY

Miss Chitnipar Sam-angsri was born on the 2nd of December 1979 in Nakhon si thammarat, Thailand. She graduated her bachelor's degree of science in biology from the Faculty of Science, Prince of Songkla University in 2001. She continued her graduated study for master's degree of science in zoology at the Faculty of Science, Chulalongkorn University in 2002.