จุลพยาธิวิทยาของตับปลาตะเพียน *Puntius gonionotus* บริเวณพื้นที่เกษตรกรรมคลอง 7 จังหวัดปทุมธานี

นางสาววธิพร เย็นฉ่ำ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสัตววิทยา ภาควิชาชีววิทยา
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547

974-53-1481-1

HISTOPATHOLOGY OF COMMON SILVER BARB *Puntius gonionotus* LIVER AT KLONG 7 AGRICULTURAL AREA, PATHUM THANI PROVINCE

Miss Watiporn Yenchum

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Zoology
Department of Biology
Faculty of Science
Chulalongkorn University
Academic year 2004

ISBN 974-53-1481-1

Thesis Title	HISTOPATHOLOGY OF COMMON SILVER BARB *Puntius gonionotus* LIVER AT KLONG 7 AGRICULTURAL AREA, PATHUM THANI PROVINCE.
By	Miss Watiporn Yenchum
Filed of Study	Zoology
Thesis Advisor	Associate Professor Kingkaew Wattanasirmkit, Ph.D.
Thesis Co-advisor	Assistant Professor Kumthorn Thirakhupt, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

…….. Dean of Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTEE

…….. Chairman
(Associate Professor Suchinda Malaivijitnond, Ph.D.)

…….. Thesis Advisor
(Associate Professor Kingkaew Wattanasirmkit, Ph.D.)

…….. Thesis Co-advisor
(Assistant Professor Kumthorn Thirakhupt, Ph.D.)

…….. Member
(Assistant Professor Patchanee Singh-asa, Ph.D.)

…….. Member
(Parkorn Varanusupakul, Ph.D.)

วธิพร เย็นฉ่ำ : จุลพยาธิวิทยาของตับปลาตะเพียน *Puntius gonionotus* บริเวณพื้นที่เกษตรกรรมคลอง 7 จังหวัดปทุมธานี (HISTOPATHOLOGY OF COMMON SILVER BARB *Puntius gonionotus* LIVER AT KLONG 7 AGRICULTURAL AREA, PATHUM THANI PROVINCE) อ. ที่ปรึกษา: รศ. ดร. กิ่งแก้ว วัฒนเสริมกิจ, อ. ที่ปรึกษาร่วม ผศ. ดร. กำธร ธีรคุปต์ 125 หน้า. ISBN 974-53-1481-1.

ในปัจจุบันมีการตระหนักถึงการใช้สารฆ่าแมลงสังเคราะห์ในพื้นที่เกษตรกรรมมากขึ้น เพราะกำจัดแมลงศัตรูพืชได้รวดเร็วกว่าสารฆ่าแมลงทางชีวภาพ สารฆ่าแมลงกลุ่มออร์กาโนฟอสเฟตถูกนำมาใช้มากในพื้นที่เกษตรกรรม ทำให้สามารถปนเปื้อนเข้าสู่ระบบนิเวศน์โดยการชะล้างจากแหล่งเกษตรกรรมลงสู่แหล่งน้ำ แต่ข้อมูลการศึกษาความเป็นพิษของสารพิษในสิ่งแวดล้อมมีน้อย ดังนั้นการศึกษาครั้งนี้มีจุดประสงค์เพื่อศึกษาจุลพยาธิวิทยาของเนื้อเยื่อตับปลาตะเพียน *Puntius gonionotus* ตรวจหาสารพิษตกค้างกลุ่มออร์การโนฟอสเฟตในน้ำบริเวณพื้นที่เกษตรกรรม คลอง 7 จังหวัดปทุมธานี และศึกษาความเป็นพิษของเอ็นโดซัลแฟนต่อตับปลาตะเพียนอายุ 4 เดือน ในห้องปฏิบัติการ ที่ระดับความเข้มข้น 0.06, 0.25, 0.50 และ 1 ไมโครกรัมต่อลิตร เก็บตัวอย่างปลาตะเพียนและน้ำบริเวณคลอง 7 ทั้งหมด 3 สถานี ตลอดความยาว 20 กิโลเมตร ระหว่างเดือนมีนาคมถึงเดือนธันวาคม 2547 นำตัวอย่างตับปลาตะเพียนมาชั่งน้ำหนักเพื่อหาค่าดัชนีความสัมพันธ์ระหว่างน้ำหนักตับและน้ำหนักตัว ศึกษาจุลพยาธิวิทยาโดยเตรียมเนื้อเยื่อตับด้วยวิธีการมาตรฐานของพาราฟิน เทคนิค ย้อมด้วยสี H&E และตัดเนื้อเยื่อแบบแช่แข็งเพื่อศึกษาฮิสโตเคมี โดยย้อมสี Oil red O และ สี PAS นำไสลด์ทั้งหมดมาศึกษาภายใต้กล้องจุลทรรศน์แบบใช้แสง ผลการวิเคราะห์ค่าดัชนีความสัมพันธ์ระหว่างน้ำหนักตับและน้ำหนักตัวของปลาจากคลอง 7 และตับปลาที่ทดสอบความเป็นพิษของสารฆ่าแมลงเอ็นซัลแฟนในห้องปฏิบัติการ พบว่ามีค่าดัชนีความสัมพันธ์ระหว่างน้ำหนักตับและน้ำหนักตัวของทั้งสองกลุ่มต่ำกว่าของตับปกติในกลุ่มควบคุมอย่างมีนัยสำคัญ ($p \leq 0.05$) ผลเนื้อเยื่อวิทยาของตับปลาจากคลอง 7 และกลุ่มการทดสอบความเป็นพิษของสารฆ่าแมลงเอ็นโดซัลแฟน พบความผิดปกติของตับปลาตั้งแต่มีความรุนแรงน้อยจนถึงความรุนแรงมาก ได้แก่ การบวมของเซลล์ตับ มีการสะสมไฮยาลิน แกรนูล มีลักษณะการตายของเซลล์เป็นกลุ่มๆ และการตายแบบกระจายรอบหลอดเลือด การคั่งของเลือดในไซนูซอยด์และเส้นเลือดแดง การอักเสบของเยื่อหุ้มตับ การหลุดออกและการหนาของชั้นเนื้อเยื่อบุหลอดเลือด มีเม็ดเลือดขาวแทรกเข้ามาบริเวณที่มีการอักเสบ และการสะสมของไกลโคเจนในตับปลาจากคลอง 7 จากการศึกษาทางฮิสโตเคมีโดยย้อมด้วยวิธี PAS พบการสะสมของไกลโคเจนน้อยลง แต่ในกลุ่มทดสอบด้วยเอ็นโดซัลแฟนพบการสะสมไกลโคเจนเพิ่มมากขึ้น จากการย้อมด้วย Oil red O พบว่ามีการสะสมไขมันในไซโตพลาซึมของเซลล์ตับของปลาจากคลอง 7 เพิ่มขึ้น ส่วนในกลุ่มทดสอบด้วยเอ็นโดซัลแฟนมีการสะสมไขมันน้อยลง ความเป็นพิษต่อตับของเอ็นโดซัลแฟนขึ้นอยู่กับความเข้มข้นของสาร จากการศึกษาสารตกค้างของยาฆ่าแมลงกลุ่มออร์กาโนฟอสเฟตในน้ำบริเวณคลอง 7 โดยวิธีแกส โครมาโตกราฟ ตรวจไม่พบสารตกค้างกลุ่มออร์การโนฟอสเฟตในแหล่งน้ำนี้

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

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

ปีการศึกษา……2547…….. ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....……..

4572465623: MAJOR BIOLOGY

KEY WORD: ORGANOPHOSPHATE INSECTICIDE / HISTOPATHOLOGY / TOXICITY / GAS CHROMATOGRAPHY

WATIPORN YENCHUM: HISTOPATHOLOGY OF COMMON SILVER BARB *Puntius gonionotus* LIVER AT KLONG 7 AGRICULTURAL AREA, PATHUM THANI PROVINCE. THESIS ADVISOR: ASSOC. PROF KINGKAEW WATTANASIRMKIT, PhD., THESIS CO-ADVISOR: ASST. PROF. KUMTHORN THIRAKHUPT, Ph.D., 125 pp. ISBN 974-53-1481-1

At present, there is growing awareness for the increasing use of synthetic insecticides in agricultural area. Organophosphate insecticides, which are mainly used in agricultural areas, often contaminate into the freshwater ecosystem by the run-off from the land and then to aquatic system. There was less information on the toxicity of affected toxicants on fish in the environment. Therefore, the objectives of this study were to assess the histopathology of *Puntius gonionotus* liver, to determine the organophosphate insecticide residues in water at Klong 7 agricultural area, Pathum Thani Province and to study the toxicity of endosulfan on the liver of 4 month old *Puntius gonionotus* at the concentration levels of 0.06, 0.25, 0.50 and 1 ppb. The fish samples and water samples were collected from three sites along 20 kilometers of Klong 7 sub-canal from March to December 2004. The liver of fish from Klong 7 groups, control group and endosulfan treated groups were prepared for histological study by paraffin technique and staining with H&E. The tissue sections for histochemical study were prepared by frozen technique and staining with Oil red O and PAS technique before the sections were observed under the light microscope. The results showed that the percent relative liver weight (%RL) of fish liver from Klong 7 and endosulfan treated groups at the concentration of 0.06, 0.25, 0.50 and 1 ppb were significantly lower than the control group ($p \leq 0.05$). The results of histopathological study of Klong 7 fish liver and endosulfan treated fish liver exhibited various lesion of changes from mild to severe, i.e. hydropic swelling, hyalin granule accumulation, foci and diffuse necrosis near the blood vessel, blood congestion in sinusoid and central vein, subcellular space inflammation, and endothelial of blood vessel ruptured and thickening. Lymphocyte and granulocyte infiltrations were seen in the inflammatory area. In the histochemical studies, the glycogen accumulation decreased in Klong 7 fish liver, but increased in endosulfan treated liver. The lipid accumulation of Klong 7 fish liver was found, but in endosulfan treated group, decreasing of lipid accumulation in cytoplasm of hepatocytes was seen. The damaged level of the endosulfan treated liver depends on the concentrations. The quantitative studies of organophosphate insecticide residues in water of Klong 7 by GC/FPD showed non-detectable result.

Department.....Biology..... Student's signature.....W. Yenchum.....

Field of study.....Zoology ..... Advisor's signature.....K. Wattanasirmkit.....

Academic year.....2004..... Co-advisor's signature.....K. Thirakhupt.....

ACKNOWLEDGEMENTS

First of all, I would like to express my deepest gratitude to my advisor, Associate Professor Dr. Kingkaew Wattanasirmkit and my co-advisor, Assistant Professor Dr. Kumthorn Thirakhupt for professionalism, discerning suggestions, guidance, forbearance, encouragement and critical reading through out the study.

I wish to express my grateful thank to Associate Professor Dr. Suchinda Malaivijitnond, chairman of the thesis committee for her advice. I also would like to express my appreciation to Assistant Professor Dr. Patchanee Singh-asa and Dr. Parkorn Varanusupakul, the thesis committee member for their valuable suggestions and comments.

I would like to thank all members of the Histology lab and GC lab for their supports, suggestions and moral supports. The cheerful willingness of my friends is truly appreciated.

I also would like to thank many people who supported in various ways during my M.Sc study.

My greatest thanks is to the Development and Promotion of Science and Technology Talents Project (DPST) who supports the scholarship of my education.

This work is supported partly by the National Research Center for Environmental and Hazardous Waste Management (NRC-EHWM).

Finally, I am eternally grateful to my beloved parents and all my dearest family members for their enormously ever-willing support. Thanks are also extended to everyone who has contributed to the success of this thesis.

CONTENTS

LIST OF TABLES

TABLES PAGE

LIST OF FIGURES

FIGURES PAGE

LIST OF FIGURES (continue)

FIGURES PAGE

CHARPTER I

INTRODUCTION

At present, there is growing awareness for the increasing use of pesticides in agricultural areas. Pesticides are mainly used for crop protection and increase production. Synthetic insecticides are popular to use because they can kill pests faster than biopesticides. Synthetic insecticides are now causing many severe problems, such as causing health hazard to human and non-target organism, being contaminated in food and causing resistance of insects to insecticides.

Organophosphates are the most popular insecticides used in cultivated area because they are unstable or non persistent. They are used as substitutes for the persistent organochlorines. In 1991, Thailand imported 9,677 tons of pesticides for the value of 1,197 billion Baht. The majority was 5,390 tons of organophosphates at the value of 588 billions Baht (DOA, 1991). Organophosphate insecticides used in agricultural areas often contaminate surface water by the run-off from the sprayed field (Nimmo, 1985), and then absorbed by the sediment or organic particles in water and flow down stream (Jaffe, 1991). Therefore, fish may be contaminated either directly or indirectly via their food. Although organophosphate insecticides can break down rapidly in the environment but the continuous use, in overdose and overtime, can have negative effect on aquatic animals especially fish. Although this effect may not kill living organisms immediately but it can decrease fitness and survival of them.

In order to understand the effect of toxicants on fish, the determination of biological effects at the early adverse alteration is necessary. The study on biological responses of fish in terms of physiological, hematological as well as histological change will provide facts concerning with change of the external environment.

In the attempt to define and measure the effect of pollutant on an ecosystem, biomarkers have attracted a great deal of interest. The principle behind the biomarker approach is the analysis of an organism' s physiological or biochemical response to pollutant exposure. Compared with chemical residues analysis, biomarkers have an advantage of being a measure of the stress incurred in the organism and so are more biologically relevant.

There is less information on the toxicity of toxicants on fish in the environment especially in the Common Silver Barb *Puntius gonionotus*, which is an economic species and widely distributed in many agricultural areas of Thailand. Klong 7, Pathum Thani Province is an agricultural area where the majority of land is a rice field and farmers cultivate rice all year. The farmers have used various insecticides in the farmland and often use in large quantity for protecting crops from pests. The repeated uses of organophosphate pesticides and the persistence of these products in the environment are large enough to contaminate even at some distance from the source of pollution (Bender, 1969).

The liver is a crucial organ, involving with the accumulation, biotransformation and excretion of toxicants. Therefore, the liver has been considered as a target organ for the study on the effect of toxicants. The histological change of the liver is an important biomarker that demonstrates the effect of environmental pollutants on organism.

Objectives

1. To assess histological change of the liver in the Common Silver Barb *Puntius gonionotus* at Klong 7 agricultural area, Pathum Thani Province.
2. To determine organophosphate insecticide residues in water at Klong 7 agricultural area, Pathum Thani Province.

Anticipated benefits

1. Obtain basic knowledge about histology of the liver of the Common Silver Barb *Puntius gonionotus.*
2. Obtain histological data of the Common Silver Barb *Puntius gonionotus* liver captured at Klong 7 agricultural area.
3. Provide data about organophosphate insecticide residues in water that can be used for the risk assessment.
4. Provide useful information to determine toxicity limits of organophosphate insecticide residues in freshwater ecosystem.

Scope of this study

1. The scope of this study is to determine the histological alteration in the liver of the Common Silver Barb *Puntius gonionotus* captured from Klong 7 agricultural area, Pathum Thani Province in two seasons, rainy season (April to October) and dry season (November to March). The control group of the Common Silver Barb at 4 month old was taken from Mongkol Farm and cultured in the laboratory for three months.
2. Water samples were collected from Klong 7 agricultural areas, Pathum Thani Province every month from March to December 2004
3. Organophosphate insecticide in the water sample were analyzed residues using eight standard species; *o,o,o*-Triethylphosphothioate, Thionazin, Sulfotepp, Dimethoate, Disulfoton, Methyl parathion, Ethyl parathion and Famphur.

CHAPTHER II

LITERATURE REVIEW

History of Insecticides

Some 10,000 species of more than 1 million species of insects are crop eating, and of these, approximately 700 species worldwide cause most of the insect damage to man's crops, in the field and in the storage. Humanoids have been on earth for more than 3 million years, while insects have existed for at least 250 million years. We can only guess, but the first materials likely used by our primitive ancestors to reduce insect annoyance were mud and dust spread over their skin to repel biting and tickling insects, a practice resembling the habits of elephants, swine, and water buffalo. Under these circumstances, mud and dust would be classified as repellents, a category of insecticides. Historians have traced the use of pesticides to the time of Homer around 1000 B.C. The earliest records of insecticides pertain to the burning of "brimstone" (sulfur) as a fumigant and use of gall from a green lizard to protect apples from worms and rot. Later, a variety of materials used were found with questionable results such as extracts of pepper and tobacco, soapy water, whitewash, vinegar, turpentine, fish oil, brine, lye and many others. At the beginning of World War II (1940), insecticide selection was limited to several arsenicals, petroleum oils, nicotine, pyrethrum, rotenone, sulfur, hydrogen cyanide gas, and cryolite. It was after the World War II that opened the Chemical Era with the introduction of a totally new concept of insect control chemicals--synthetic organic insecticides, the first of which was DDT (George, 1999).

Organophosphates

Insecticides fall into two types; inorganic and organic compound. The organic insecticides are separated into the following main groups: organochlorines, organophosphates, carbamates, and other groups such as pyrethroid and Pyrethrum.

Organophosphates (OPs) are the currently used generic term that includes all insecticides containing phosphorus. Other names used, but no longer in vogue, are

organic phosphates, phosphorus insecticides, nerve gas relatives, and phosphoric acid esters. All organophosphates are derived from one of the phosphorus acids, and as a class are generally the most toxic of all pesticides to vertebrates. Because of the similarity of OP chemical structures to the "nerve gases", their modes of action are also similar. Their insecticidal qualities were observed in Germany during World War II in the study of the extremely toxic OP nerve gases sarin, soman, and tabun. Initially, the discovery was made in search of substitutes for nicotine, which was heavily used as an insecticide but in short supply in Germany. The OPs have two distinctive features: they are generally much more toxic to vertebrates than other classes of insecticides, and most are chemically unstable or nonpersistent. It is this latter characteristic that brought them into agricultural use as substitutes for the persistent organochlorines (George, 1999).

Mode of action: The OPs are tying up or inhibiting certain important enzymes of the nervous system, namely *cholinesterase* (ChE). The enzyme is phosphorylated when it becomes attached to the phosphorous of the insecticide, a binding that is irreversible. This inhibition results in the accumulation of acetylcholine (ACh) at the neuron/neuron and neuron/muscle (neuromuscular) junctions or synapses, causing rapid twitching of voluntary muscles and finally paralysis (George, 1999).

All OPs are esters of phosphorus, having varying combinations of oxygen, carbon, sulfur and nitrogen attached, resulting in six different subclasses: phosphates, phospho-nates, phosphorothioates, phosphorodithioates, phosphorothiolates and phosphoramidates. These subclasses are easily identified by their chemical names. The OPs are generally divided into three groups; aliphatic, phenyl, and heterocyclic derivatives.

Aliphatics: The aliphatic OPs are carbon chain-like in structure. The first OP brought to agriculture; TEPP (1946) belongs to this group. Other examples are malathion trichlorfon (Dylox®), monocrotophos (Azodrin®), dimethoate (Cygon®), oxydemetonmethyl (Meta Systox®), dimethoate (Cygonr®), dicrotophos (Bidrin®),

disulfoton (Di-Syston®), dichlorvos (Vapona®), mevinphos (Phosdrin®), methamidophos (Monitor®), and acephate (Orthene®).

Phenyl derivatives: The phenyl OPs contain a phenyl ring with one of the ring hydrogens displaced by attachment to the phosphorus and other hydrogens frequently displaced by Cl, NO_2, CH_3, CN, or S. The phenyl OPs are generally more stable than the aliphatic, thus their residues are longer lasting. The first phenyl OP brought into agriculture was parathion (ethyl parathion) in 1947. Examples of other phenyl OPs are methyl parathion, profenofos (Curacron®), sulprofos (Bolstar®), isofenphos (Oftanol®), Pryfon®, fenitrothion (Sumithion®), fenthion (Dasanit®), and famphur (Cyflee®), Warbex®.

Heterocyclic derivatives: The term heterocyclic means that the ring structures are composed of different atoms, e.g. oxygen, nitrogen or sulfur. The first of this group was diazinon introduced in 1952. Other examples in this group are azinphos-methyl (Guthion®), azinphos-ethyl (Acifon®), Gusathion®), chlorpyrifos (Dursban®), Lorsban®, Lock-On®, methidathion (Supracide®), phosmet (Imidan®), isazophos, Brace®, Triumph®), and chlorpyrifos-methyl, Reldan®.

Mainly, insecticides are to produce healthy food, affordable for consumers to purchase, while ensuring that farmers are able to earn a decent income. Recently, with greater environmental awareness in society in general, it is also now very important to protect the agricultural environment. This is a big challenge for farmers as they must still obtain reasonable yields and produce quality product in order to meet the demands of the market. Both these can be severely affected by harmful organisms, commonly referred to as pests (weeds, disease, etc.) that compete against, infect or damage the cultivated crop in a detrimental manner. The most economic and effective way to handle these has been to employ pesticides, many of which are now composed of synthetic chemicals, and it is these substances, very beneficial from the economic and production aspects of farming, which can pose risks to human health and the environment if not properly used. Pest problems are not new; in fact, they have been around as long as agriculture itself. But the pest pressure faced by farmers is now as great as it ever was:

the world's fast-growing human population needs to be fed from an always shrinking base of agricultural land, and the substantial damage that can be inflicted by pests (e.g. insects, diseases, weeds, rodents, birds) on crops is the margin between a good harvest and a bad one (Thomson, 1995). Pests can reduce the quality of a harvest as well as its quantity. Since the quality of food is increasingly important to consumers, a pest could reduce the value of a crop. Hence, crop protection has always been an important component of agriculture, leading to the development and employment of measures that can limit damage, such as synthetic chemicals. Easily stored for long periods in a compact form, easily applied at very short notice (provided the machinery is available and the weather conditions are suitable), they are fast acting and efficient. They can also be toxic, and the farmer must use pesticides wisely to make sure that they will not harm the applicator, the farm family and the surrounding environment.

The chemical insecticides have various disadvantages. The first, insect is resistance to insecticides and rapidly increases its number, severely destroys crop products. Second, chemical insecticides have residues in environment and these residues contaminate environment that may effect to non-target organisms on nature enemies and pollinators or other animals in ecosystem, especially aquatic system because most agricultural area is near to the fresh water steams, lakes, ponds and rivers which is easily contaminated to surface water by run off from the land or is absorbed onto river sediment or organic particles in water, down stream from sprayed fields (Jaffe, 1991). When the insecticide compounds contaminated in aquatic system and food web, aquatic animals may be exposed to this toxic compound directly by water and indirectly by food. Chemical insecticides can accumulate in the body of organisms at higher tropic level and the most in top carnivore. Human is top carnivore, therefore human has high risk to be contaminated by toxic compound.

Almost all of the pesticides used in Thailand are imported. Pesticides imported to Thailand have increased rapidly over the past decade (Department of Agriculture, 1996). In Thailand, more than 14,996,297 kilograms (3,136,144,282 Bath) of insecticides

were imported in 2003. In 1996, mainly insecticides imported were organophosphates 5,562,460 kilograms (637,197,259 Bath), calculated from 38.68% of total insecticides imported. Types of insecticides imported in 1993 to 1996 were mainly organophosphates. High-level import in every year has been monocrotophos, methamidophos and parathion methyl (Department of Agriculture, 2003).

Water bodies such as streams, lakes and rivers are polluted by many kinds of pesticides. In 1985-1988, Department of Agriculture (DOA) collected and analyzed water samples in agricultural areas throughout Thailand. The results showed that organochlorine insecticides were the most common pollutants in water. Although organophosphates are easily degraded or unstable in the environment but sometimes the farmers use than in large quantity. These compounds in the aquatic system may be persistent in the environment long enough and can damage organism. Chumraskul et al. (1997) reported that organophosphate residues in water and sediment from the Ta-Jean River and the tributary were found in 89 % and 72.7 % of the total sample, respectively. In the water, monocrotophos, dicrotophos, diazinon, dimethoate, methyl parathion, malathion and fenitrothion in the level of 0.01 to 0.57 ppb. Were detected. In the sediment, diazinon, methyl parathion and fenitrothion were found. Monocrotophos is highly hazardous and was banned for agricultural use since 2000. Sakulteangtong et al. (1996) examined organophosphate residues from the Joa-Praya River and found malathion, parathion methyl, monocrotophos, and chlorpyriphos ethyl in water sample. In the sediment, malathion, dimethoate, monocrotophos and parathion methyl were found. Chumraskul et al. (1997) studied organophosphate residues in Pa-Sak River. They found organophosphate residues in 15.66 % of the total sample. Monocrotophos, diazinon, methamidophos, dimethoate, chlorpyriphos ethyl, pirimiphos methyl, methyl parathion, profenophos, fenthion, ethiom and azinphos ethyl were found in level between 0.01 to 0.70 ppb. In the sediment, 2.68 % of the total sample were found. They were dicrotophos, diazinon, methamidophos, ethion, chlopyriphos, and phosphamidon at the level of 0.003 to 0.012 ppm. Later, chumraskul et al. (1997) determined organophosphate residues from Bang-Pa-Kong River and its tributary and found

organophosphate residues in water and sediment about 50.8 % and 11.1 % of the total sample, respectively. In the water, monocrotophos, methamidophos, diazinon, dimethoate, methyl parathion, fenitrotion and chlorpyriphos ethyl were found. In the sediment, only methyl parathion and chlorpyriphos ethyl were detected. Organophosphate residues in the water were found between 0.01 to 0.13 ppb whereas in the sediment, there were 0.01 to 0.34 ppm. Chumraskul et al. (1997) studied the distribution of pesticides in water and sediments in Mae Klong River and tributaries. The results indicated that seven species of organochlorines; BHC, heptachlor, aldrin, dicofol, endril, endosulfan and DDT and seven species of organophosphates; monocrotophos, methamidophos, diazenon, dimethoate, methyl parathion, malathion and chlopyrifos ethyl were found. Carbamates; cabufuran and metalaxyl were detected. Pyrethroid insecticides; cypermethrin and fenvalerate were noticed. In the sediments, organochlorines same in water except endosulfan, organophosphate; monocrotophos, dicrotophos, dimethoate, methyl parathion, fenitrithion and pirimiphos methyl were found. Carbamates and pyrethroid insecticides were not detect. Sakulteangtong et al, (2001), monitored agricultural toxic substances in the water and sedient samples from Pakpanang River Basin, found 60% of organochlorines total in the sample. There were DDT, dieldrin, heptachlor, aldrin and dicofol. Organophosphates, carbamates and pyrethroid insecticides were not detected. In sediment, organochlorines 46.7% of the total samples; DDT, endosulfan, dieldril and dicofol and organophosphates; chlorpiriphos methyl, dimethoate, malathion, parathion, profenofos, methyl parathion and chlorpiriphos ethyl were found. Carbamates and pyrethroid insecticides were not detected.

The geographic and climatic conditions make Thailand suitable for cultivating crops especially in central sector of the country is mainly agricultural area, having various crop products for example rice, maize, sugarcane, tapioca and tropical fruits, mango, mangosteen, rambutan and durian. The Rangsit great plain is one of the cultivated areas in Pathum Thani Provine. The development of the great plain had initiated in 1890, in the reign of King Rama the V, in order to increase the rice-growing

area for more export of rice production. This development the canal excavation on 24th February 1890. The mission launched by digging up the new main canal on 9th March 1890, starting from the bank of Choa Phraya River at Ban Mai subdistrict, south of Koh Yai, Pathum Thani town to Nakhon Nayok River at Plakod Hua Kwai subdistrict, Nakhon Nayok town, which was itself 12 m wide and 56 Km long; later on this canal had been widened to 16 m. King Rama the V named this canal "Rangsit-prayulasakdi", honouring Prince Rangsitprayulasakdi (NSM, 2001). After the completion of the excavation of Rangsit canal, most of the branch canals and side canals; the people have moved in great numbers until the large community of great wealth in rice production and local trade were formed. Being provided with enough surface water all year round. 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. Its main portion comprises more or less waterlogged fields, and marshes that are suitable habitats for many forms of aquatic lives for their living, spawning and nursing young in the extremely plentiful stocks of natural foods. Canals; from the irrigation projects both Choa Praya and Nakhon Nayok Rivers, the water will be distributed through the connecting canals to nourish human communities and agricultural areas; with the Rangsitprayulasakdi canal as a main canal and other smaller ones branching out from it. Fish is the readily available and cheap protein source that can be taken easily along the canals and in the waterlogged fields. The local fisherman utilizes many kinds of fishing gears, according to the suitability of the site and the desired fish species. The stationary fishing gears, seen frequently along the canals, include the large sized lift net or gill net for catching small slender barbs to large ones and the feather backs; also including various types of traps such as the standing traps fixed together with drift fences, and set in parallel lines along the canal banks for catching snakehead, climbing perch, etc. (NSM, 2001).

Aquatic Ecosystem

The aquatic environment is complex and diverse. It includes several distinct ecosystem types (freshwater streams, lakes, ponds and rivers; estuaries, marine coastal and deep ocean water) with many different biotic and abiotic components. The biotic or living components consist of many combinations of plants, animals and microorganisms that inhabit specific ecological niches in each ecosystem. The abiotic or nonliving components include the physical environment within the boundaries of the ecosystem. Each aquatic ecosystem is thus a product of complex interaction of living and nonliving components. Since ecosystem involves complex interaction of physical, chemical and biological factors. It is difficult to understand the response of the system to chemicals unless the relationships among components are well defined. Moreover, similar ecosystems are not necessarily affected in the same response by contamination of the same chemical. Minor difference in the physical, chemical properties and biological composition can result in different ways to the ecosystem. (Rand and Petrocelli, 1985)

Aquatic Toxicology

Aquatic toxicology has been the qualitative and quantitative study of the adverse or toxic effects of chemicals and other anthropologenic materials or xenobiotics on aquatic organism (Rand and petrocelli, 1985). Toxic effects may include lethality and sub lethal effect, for example, change in growth, development, reproduction, pharmacokinetic responses, pathology, biochemistry, physiology and behavior. The quantifiable criteria may be used for monitoring the effects such as change in percent relative weight, number of hepatocytes damage, and accumulation of lipid droplet. The toxicants enter aquatic ecosystem from (1) non-point sources such as agricultural runoff from land, contaminated ground water and bottom sediments, urban runoff and atmospheric fallout and (2) point sources such as discharges (effluents) from manufacturing plants, hazardous waste dispersal sites and municipal waste water treatment plants. The most innocuous chemical substances can have undesirable or distinctly harmful effects when taken up by an organism in sufficient amounts. On the other hand, if minute quantities of toxic substances are uptake, it can result in no

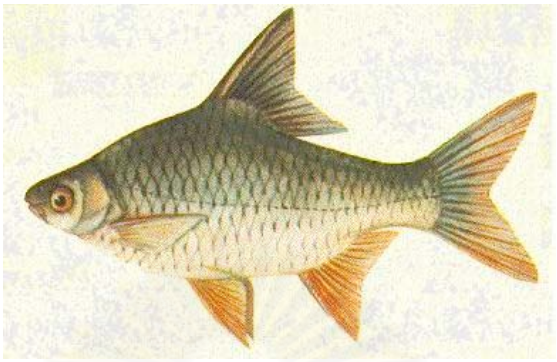
apparent adverse effect. Therefore, it is a vital concept in toxicology that in general “no substance is completely safe and no substance is completely harmful” (Rand and Peteocelli, 1985). The factor that determines whether a chemical agent is potentially harmful or safe is the relationship between the concentration and the duration of exposure. In the aquatic environment the concentration, transport, transformation and disposition of chemical are primarily controlled by (1) the physical and chemical properties of the chemical compound, (2) the physical, chemical and biological properties of the ecosystem, (3) the source and input rate of the chemical into the environment (Rand and petrocelli, 1985).

The major reason for carrying out toxic test with fish and other aquatic organisms is to determine which concentrations of a substance are harmful to the organism and which have no apparent effect. The endpoints that have been considered in tests to determine the adverse effects of toxicants, including death and survival rate, decrease in reproduction and growth, locomotors activity, blood chemistry and histophatology. From the results, toxicants can recommend maximum concentration for the well being of aquatic organism, engineers can design treatment systems to achieve desired levels and fisheries manager can evaluate chemical measurements in local bodies of water (Sprague, 1990).

Adverse effects may be produced by acute or chronic exposure to chemicals or other potentially toxic agents. In general, an acute exposure concerns a short period of time compare to the life cycle of an organism, the test last about a week or less for fish. The effect usually occurs within 4 days. Mortality is the end point. On the other hand, a chronic exposure may involve the entire reproductive life cycle. Exposure that are intermediate in duration, a month to several month, are less than a complete reproductive life cycle and include exposure during sensitive early stages of development. In mammalian toxicology, it usually signifies exposures lasting one – tenth of a lifetime or longer. Therefore, it is sometime used to mean a full life cycle test in aquatic toxicology. (Schreck and Moyle, 1990)

All the data provided by toxicity potential is used for determine compliance with permit toxicity limits, to aid in the development and implementation of toxicity reduction plans and for risk assessment. Furthermore, the data of toxicity test may be assembled to derive water quality criteria, to monitor the toxicity of chemicals and effluents and to evaluate the quality of surface water. Using fish to assess the quality of an effluent is an economical and meaningful procedure, especially if many waste substances are present of if it is not known exactly what is present, such tests could involve monitoring a cage of fish placed in a river below an industrial outfall or periodic standardized toxicity tests of chemical or effluent (USEPA, 1994).

Fish, like mammals, are susceptible to a variety of environmental stressors that may directly cause, or indirectly predispose, them to develop different types of lesions. These stressors may be biological (bacterial, viral, fungal, parasitic), chemical (pollutants, toxins, suboptimal water quality, hormonal changes due to photoperiod or breeding) and/or physical (rapid water temperature change, trauma). Disease outbreaks and mortality occur naturally in all wild populations. What causes concern, however, is when huge numbers (i.e., hundreds of thousands) of fish exhibiting lesions, morbidity, or death occur in a relatively with duration of expose and dose of toxicants.



Common Silver Barb *Puntius gonionotus*

Taxonomy

Phylum Vertebrata

Class Actinopterygii

Order Cypriniformes

Family Cyprinidae

Genus *Puntius*

Species *Puntius gonionotus*

Common name : Common silver Barb

Thai common name : Pla Ta Pien

Scientific name : *Puntius gonionotus*

Synonyms : *Puntius viehoeveri, Puntius javanicus, Barbus gonionotus, Barbonymus gonionotus*

Habitat and Biogeography: Common Silver Barb is found in the tropic, 24°N - 8°S. This fish is widely distributed in southeast Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Java (Chan Sokheng, 1999). It occurs throughout the whole stretch on the Mekong, from the delta around the saline intrusion zone to Chiang Khong in Thailand (Kottelat, 1998). This fish occurs in freshwater, found at midwater to bottom depths in rivers, streams, floodplains, and occasionally in reservoirs. It seems to prefer standing water habitats instead of flowing waters.

Biology and Characteristic: The Common Silver Barb has dorsal spines (total): 4-4; dorsal soft rays (total): 8-8; anal spines: 3-3; anal soft rays: 6-7. Body is strongly compressed. The back is elevated. Its dorsal profile arched, often concave above the occiput. The head is small, the snout is pointed and the mouth is terminal. The barbels are very minute or rudimentary, especially the upper ones, which sometimes disappear entirely. Color when fresh is silvery white, sometimes with a golden tint. The dorsal and caudal fins are gray to gray-yellow; the anal and pelvic fins are light orange, their tips reddish; the pectoral fins are pale to light yellow (Taki, 1974). Very few tubercles on the snout that are not visible without magnification, snout length much less than the width of the eye socket. Anal-fin was 6-7 branches rays (Rainyboth, 1996). It inhabits the flooded forest during high water period and feeds on plant matter (e.g. leaves, weeds, *Ipomea reptans* and *Hydrilla*) and invertebrates (Mohsin and Ambak, 1983). It is a migratory species but not considered to be a long-distance migrant. Regarded as local migrant, this species moves from the Mekong up into small streams and canals and onto flooded areas during the rainy season and back again during receding water (Chan Sokheng, 1999). Some reports indicated that the first rainy and rising water levels trigger upstream migration of this fish. When it finds a tributary, canal or stream it moves upstream and eventually onto flooded areas. When water recedes, it migrates back into canals and streams and into the Mekong again (Chan Sokheng, 1999). It is useful in cropping excessive vegetation in reservoirs (Davidson, 1975). A specimen measuring 45 cm, total length (2,100 g) was reportedly caught from Dan Tchang Reservoir, Thailand on 8 July, 2003.

The reason of selecting Common Silver Barb *Puntius gonionotus* as test organism in this study as follows;

1. Widely available and abundant in Thailand
2. Good represent of he aquatic ecosystem that may receive the impact
3. Ecological and commercial importance

The effect of toxicants on fish

Effect of insecticides

Tolga and Serap (2002) reported about micronucleus formation in fish erythrocytes, as an indicator of chromosomal damage. Nucleolar organizer regions (NORs) stained with colloidal silver techniques indicate sites of active RNA transcription. The number and size of NORs in interphase nuclei reflect cellular activities such as proliferation and differentiation of cells. In this study, nuclear (micronucleus frequency) and nucelolar (changes in quantitative characteristics of nucleoli) biomarkers were used to evaluate the functional and structural genotoxic effect of the pyrethroid insect lambda-cyhalothrin on *Garra rufa* (Pisces: Cyprinidae). Chlorpyrifos (CPF), potent acetyl cholinesterase inhibitor, interferes with neurobehavioral development. They found that early developmental CPF exposure caused behavioral alterations in zebra fish, which lasted throughout adulthood. The molecular mechanisms by which early developmental CPF exposure produces these behavioral impairments expressed in adulthood can now be studied in the zebra fish mode (Edward, 2004). The impact of a sublethal concentration of a endosulfan on the activity, specific activity, electrophoretic patterns and kinetic properties of crude and purified lactate dehydrogenase (LDH) from the liver and the skeletal muscle of the freshwater catfish, *Clarias batrachus,* was evaluated. The endosulfan significantly reduced the activity and the specific activity of liver and muscle LDH but had no effect on total protein content. The inhibition pattern of the liver LDH remains unaltered, whereas it is modulated by endosulfan in the case of muscle LDH. These results demonstrate that endosulfan inhibits liver and muscle LDH through enzyme-endosulfan complexing (Rajnikant and Shukla, 1996). The effect of carbofuran on certain metabolites and enzymes of protein and carbohydrate metabolism was evaluated in liver and muscle tissues of the freshwater fish, *Clarias batrachus.* Total protein showed a delayed decrease in liver and muscle tissues but recovered by the end of the recovery period. Free amino acid content was affected little in liver, but was elevated in muscle, and ammonia levels were elevated in both tissues throughout the exposure period, and ammonia levels in liver remained elevated during recovery.

Glycogen content of liver declined substantially, and rebounded after transfer of fish into clean water. The activity levels of alanine aminotransaminase, aspartate aminotransaminase, glutamate dehydrogenase and glycogen phosphorylase were found to increase in both tissues during the exposure period. The glycogen phosphorylase an activity in liver was suppressed on exposure to carbofuran. The enzymes exhibited different recovery pattern in liver and muscle tissues of *C. batrachus* (Ghousia, 2002). Fenitrothion, as an organophosphothionate insecticide was determined on the adult peppered corydoras (*Corydoras paleatus*). The static test method of acute toxicity test was used. Data obtained from acute toxicity tests were evaluated using the Probit Analysis Statistical Method. The 96 h LC_{50} value for peppered corydoras was estimated as 3.51 mg/l (Rabia, 2003). *Heteropneustes fossilis* were subjected to 5.76 and 1.44 μg/L of cypermethrin for short- and long-term experiments, respectively. Ultimobranchial glands were fixed for histological studies. Plasma calcium levels of fish exhibit a decrease after 48, 72, and 96 h. After 96 h a decrease in the staining response of the cytoplasm of ultimobranchial cells has been noticed. The nuclear volume of these cells undergoes a slight decrease. Chronically exposed fish exhibit a decrease in calcium level on day 7, which persists through 28 days. After 21 days, nuclear volume of the ultimobranchial cells undergoes a decrease and these cells exhibit a slight decrease in the staining response of the cytoplasm. Following 28 days exposure, the nuclear volume undergoes further decrease and degeneration and vacuolization sets in (Diwakar, 2003).

Effect of herbicides

Chronological effect produced by 0.17 mg atrazine in the liver of juvenile grey mullets (*Liza* sp.) were investigated increase in the size of lipid droplets and lipofuscin granules and liver lipoid degeneration (Biagianti and Bastide, 2000). The inhibition of both total and specific acetyl cholinesterase activities was measured in the whole eyes of the yellow eel *Anguilla anguilla* after exposure to the carbamate thiobencarb. The pesticide induced significant inhibitory effect on AChE activity ranging from 35% in total AChE activity to 75% in specific AChE activity (Sancho, 2002). Acute and subacute

toxicity of the herbicides trifluralin on fish was investigated that a decrease in relative growth rate was found. An increase of functional enzyme activities in blood serum and the organs examined, particularly in the highest concentration of trifluralin indicated changes in the vital organs, and was confirmed by histological analysis. The most severe changes (although mostly reversible) were found in the gills and kidney of the fish examined (Vesna and Vesela, 2002). To define targets of thiobencarb embryo toxicity and to determine the degree of protection afforded by the chorion, medaka (*Oryzias latipes*) embryos were exposed under static nonrenewal conditions**.** Liver histologic alterations were seen in chorionated embryos at EC_{50} levels and higher. Stage-specific toxicity was evident; nevertheless, the EC_{50} and NOEC values for embryos treated at stage 10 and stage 23 were similar (Alex, 2000). Asoprim and Avans (2001), on pronephros histology and ultrastructure and on activity of immunocompetent polymorphonuclear and mononuclear cells in European catfish (*Silurus glanis*). Results showed that Asoprim and Avans decreased phagocytic and intracellular killing activity of pronephros phagocytes. Morphological study indicated changes in pronephros, one of the most important haemopoietic organs in fish (Szarek, 2000). Carp (*Cyprinus carpio*) were exposed by emersion in Roundup™electron microscopy revealed that the herbicides caused appearance of myelin-like structures in carp hepatocytes, swelling of mitochondria and disappearance of internal membrane of mitochondria and mitochondria necrosis (Szarek, 2000).

Effect of fungicides

Newly hatched medaka were exposed to aqueous solutions of vinclozolin (2500 μg/l) and the vinclozolin fungicides formulation, Ronilan® (1000 and 5000 μg/l) and cyproterone acetate (1 and 10 μg/l), for 3 months. Histological evaluation of the gonadal tissues of exposed fish indicated that induced a low incidence of intersex, affected spermatogenesis in males, and induced moderate ovarian atresia. The results of this study indicate that antiandrogens have the potential to alter testicular development and gametogenesis in fish (Yiannis, 2003). A dicarboximide fungicides has a highly specific action, with a capacity to cause oxidative damage through production of free oxygen

radicals (ROS), evaluation of its capacity to induce oxidative damage in an aquatic organism such as the rainybow trout (*Oncorhynchus mykiss*) was considered of particular interest. These results suggest that iprodione is able to produce oxidative damage in primary cultured fish hepatocytes It is also well known that ROS production in fungi is due to interaction with the flavin enzyme NADPH cytochrome *c* reductase to the extent that the normal electron flow from NADPH to cytochrome *c* is blocked (Sonia, 2001). It has been thoroughly established that the vinclozolin and the persistent DDT metabolite *p*,*p*-DDE, can function as antiandrogens. In the study, juvenile guppies (*Poecilia reticulata*) were fed sublethal doses of vinclozolin, *p*,*p*-DDE or flutamide from birth to adulthood. All three chemicals caused a reduction in the orange display coloration, inhibited gonopodium development, reduced the sperm count and suppressed courtship behaviour, in a manner consistent with antiandrogen action (Mark, 2002). The chronic toxic effect of a prolonged exposure of Emisan (methoxy ethyl mercuric chloride: MeEHgCl), on the histophysiology of liver in adults and young (yearlings) of *Channa punctatus showed* cellular damage, a marked reduction in hepatosomatic index, levels of total protein and lipid, and an elevation in cholesterol and acid and alkaline phosphatase contents were recorded. However, these alterations were more pronounced in young than in adult fish (Raj Narayan Ram and Sathyanesan, 2003).

Effect of metal

Study the effect of copper exposure on swimming performance and gill-binding characteristics of wild yellow perch (*Perca flavescens*). Yellow perch from the contaminated lake also had higher resting levels of muscle glycogen and greater lactate production during high intensity exercise compared to yellow perch from the reference site. Acclimation occurred in the metal contaminated yellow perch, as seen by the significantly elevated time to death (LT50) during an acutely lethal challenge to 600 μg Cu/l (Lisa, 2004). The effect of lead (Pb) on ALA-D activity, metallothionein (MT) levels, and lipid peroxidation in liver, kidney, and blood of the toadfish *Halobatrachus didactylus* were investigated the progressive decrease of MDA concentration in the liver

and the lack of a clear induction in kidney. The histological and histochemical results demonstrated degenerative effect of lead accumulation on the tissues and the activation of lysosomal responses to induced stress (Olivia, 2003). Rainybow trout (*Oncorhynchus mykiss*) and yellow perch (*Perca flavescens)* have a different sensitivity to cadmium (Cd) in vivo (trout<LC50<perch). Adrenocortical cells were exposed to Cd for 60 min, and then stimulated with ACTH, dbcAMP or with pregnenolone, a cortisol precursor. Cd inhibited ACTH-stimulated cortisol secretion in a dose-dependent manner in both fish species, however, the EC50s (concentration resulting in 50% inhibition of cortisol secretion) was significantly lower in trout (EC50=0.09 mM) than perch (EC50=0.26 mM). Adrenocortical cells of trout were more sensitive than those of perch and Cd had a higher endocrine-disrupting potential and specificity in trout than in perch (Alexandra, 2004). In this study 96-h LC_{50} value of cadmium chloride ($CdCl_2 \cdot H_2O$), a metal salt widely used in industry, was determined for the guppy (*Poecilia reticulata*, Pallas, 1859). A 96-h LC_{50} value for *P. reticulata* was found to be 30.4 mg/l in a static bioassay test system. The behavioral changes observed in fish were, swimming in imbalanced manner, capsizing, attaching to the surface, difficulty in breathing and gathering around the ventilation filter (Mehmet, 2004). The effect of a NiO precoat on the interfacial microchemistry and the structure of gas bubbles at the steel–enamel interface were investigated using scanning electron microscopy (SEM). The resulting increase in FeO concentration reduces the viscosity of enamel. The apparent decrease in the viscosity of liquid enamel, along with formation of substantial quantities of CO and/or CO_2 gases control the distribution of gas bubbles in the enamel layer. The investigation also explains the reason for the association of large gas bubbles with Fe–Ni metal rich particles with a dendritic appearance (termed 'dendrites in the following text) at the enamel–steel interface. The role of these Fe–Ni metal rich 'dendrites' in reducing the tendency for hydrogen flaking or cracking of the enamel layer, generally referred to as fish scaling, is also elucidated (Yang, 2003). Rainbow trout (*Oncorhynchus mykiss)* were exposed to 1.65 μM of waterborne copper for 24 h. fish were then transferred to metal free water. Metallothionein mRNA induction in rainbow trout liver and gill tissue, hypoxia-inducible factor-1 (HIF-1α) accumulation in gill tissue and arithmetic mean thickness of

gill epithelium (H_{ar}) were determined at 4 and 24 h of exposure as well as 48 h after transfer to metal free water. The arithmetic mean distance from water to blood was significantly elevated after both 4 and 24 h of exposure (H_{ar} was 4.67 and 4.66 μm, respectively in exposed fish, compare to 3.81 and 3.62 μm for the corresponding control fish). During the 48 h recovery H_{ar} returned towards the control values; the recovery value of 4.21 μm was significantly lower than values during exposures. There was also a significant increase in gill metallothionein mRNA levels after the 4 h exposure with MT/GAPDH ratio of 1.288 versus the control value of 0.988. In liver, metallothionein induction was not observed. HIF-1α protein showed an increased accumulation in gills after 4 h, with the HIF-1α/α-tubulin ratio of 0.562 being significantly higher than the 24 h exposure value of 0.232. These results suggest that exposure to copper for four hours causes hypoxia in the gill epithelium, which is adequate for the activation of HIF-1α (Dalene, 2004).

Fish Liver

The liver is a largest of the extramural organs. It is roughly J-shaped, situated ventral to the esophagus and conforming to the peritoneal cavity and surrounding viscera. The color varies from dark brown to cream or even yellow. Functions of the liver include assimilation of nutrients, production of bile, detoxification, hematopoiesis, and effete red cell destruction. Parenchyma of the liver is contained within a thin capsule of fibroconnective tissue. Often the capsule is not distinguishable in light microscopic preparations. The parenchyma itself is primarily composed of polyhedral hepatocytes typically with central nuclei. Vacuolization of hepatocytes resulting from glycogen and/or fat storage can produce considerable histological variability. Other cell types typically found in liver parenchyma include hematopoietic tissue and macrophage aggregates. Venous blood enters the liver caudally from the intestine via the hepatic portal veins and branches into capillaries known as sinusoids. After passing through the sinusoids and collecting in central veins the blood exits the liver via the hepatic veins eventually returning to the heart via the sinus venosus. Sinusoids are lined with reticuloendothelial cells, which are in turn lined with hepatocytes. Adjacent sinusoids are separated from

one-another by at least two hepatocytes. In the case of glycogen vacuolization, the nuclei and cytoplasm of hepatocytes are compressed eccentrically toward the sinusoidal spaces. Bile ducts also occur within the parenchyma of the liver. Originating between adjacent hepatocytes, bile canaliculi anastomose to produce ducts of increasing diameter. Eventually the ducts merge to form the common bile duct. Smaller ducts within the liver are lined with a single layer of cuboidal epithelial cells. Larger ducts may incorporate a layer of connective tissue and thin muscularis. By the time the common bile duct exits the liver it is composed of the four basic layers of the digestive tract; mucosa (columnar epithelium), submucosa (loose connective tissue), muscularis (circularis and longitudinale), and serosa (mesothelium). The sensitivity of a species in responding to a range of contaminants is a vital determinant for its usefulness as a bioindicator. Within individuals of a bioindicator species, careful analysis will reveal biomarkers as alterations in structure and function of specific organs, tissues and cell as a consequence of prior exposure to contaminants (Jau shin, 2003).

The liver of vertebrate not only represents an organ central to numerous vital functions in basic metabolism, but it is also a major site of accumulation, biotransformation and excretion of xenobiotic compound. Thus, hepatocytes may be expected to be primary target of toxic lesions. Selection of liver cell as appropriate targets should therefore provide an opportunity for detection of suitable biomarker of environmental pollution. It is essential to realize that any physiological and biochemical change, if severe enough and protracted, will eventually result in morphological effect, structural modifications will ultimately be followed by functional consequences. Ultrastructural alterations of fish hepatocytes have repeatedly been used as monitor systems or sublethal effects of organic contaminants. However, the biomarker concept has specially been applied to particular hepatocellular changes (Braunbeck and Volkl, 1991).

Histological Alteration of fish liver

Cells are active participants in their environment, constantly adjusting structure and function to accommodate changing demands and extracellular stresses and intracellular milieu within a relatively narrow range of physiologic parameters – they maintain normal homeostasis. As cells encounter physiologic stresses or pathologic stimuli, they can undergo adaptation, achieving a new steady state and preserving viability. The principle 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 stable baseline; however, with severe or persistent stress, irreversible injury result, and the affected cell die (Eduardo and Rogerio, 1999).

Stages and characteristic of cell injury

Trump and Ginn (1969) study cell injury that changes in structure and organelles of cell after cells encounter physiologic stresses or pathologic stimuli. This hypothesis was known Final Common Pathway. 7 stages of cell injury is:

Stage 1 stage of normal cell

- normal cell membrane and organelles membrane.
- ribosome attach with RER.
- mitochondria have electron dense granule in matrix.
- normal lysosome.
- chromatin distribute through in nucleus.
- free ribosome in cytoplasm.

Stage 2 stage of after encounter stresses

- dilation of endoplasmic reticulum and some area ribosome detachment from RER.
- decrease electron dense granule in matrix of mitochondria.

- decrease glycogen granule in cytoplasm.
- chromatin dense.

Stage 3

- inner compartment of mitochondria is dense effect on darker of matrix and space of inner membrane and outer membrane is larger.
- cell membrane bleb are formed and microvilli are lost.

Stage 4

- inner compartment of mitochondria is swelling but someone is contracting or swelling and contracting in one.

Stage 5

this stage is irreversible change or point of no return

- all mitochondria is swelling, inner compartment is larger and outer membrane leak.
- in matrix of mitochondria is flocculent densities.

stage 6

- karyolysis
- eosinophilic cytoplasm
- lysosomal enzyme leak and autolysis of organelles
- ribosome detachment
- cell membrane and nuclear membrane rupture

stage 7

- inclusions in cytoplasm
- found myelin figure that homogenous material comprise of cholesterol and phospholipid.
- Karyolysis

The most frequently encountered types of degenerative changes are those of;

- hydropic degeneration
- cloudy swelling
- vacuolation
- focal necrosis
- pyknosis, karyolysis and karyorrhexis
- fatty degenerative changes
- zonal, massive and pericentral necrosis
- cirrhosis
- malignant hepatoma
- laminar or subcapsular necrosis

In general hepatocyte swelling, pyknosis of nuclei and cytoplasmic vacuolation are also commonly found in toxic condition. Acute and extensive necrosis of hepatocytes many occur in toxic conditions but focal necrosis is more common. Zonal necrosis of fish liver is difficult to ascertain because the fish liver is very much more diffuse (Roberts. 1978). Zonal and massive necrosis is rarely observed in the fish liver. However, focal hepatic necrosis is a regular lesion in which cause by the virus disease of salmonids and channel catfish. Pericentral necrosis has been reported in trout and catfish that received relatively high dose of CCl_4 or MCB (Gingerich et al., 1977). The diffuse focal necrosis is commonly observed in CCl_4 acute toxicity studies and subcapsular necrosis is also found (Gingerich et al., 1977).

Histophatological of fatty degenerative, extremes cases of the hepatocytes are shown distention of every liver cell by a single large globule of fat and where there is breakdown of liver cell membrane, macrophage invasion with ceriod or lipofuscin deposition also occurs (Roberts, 1978). The accumulation of lipophilic vacuoles had been observed in a number of fish species following experimental intoxication with

polychlorinated biphenyls (Hacking et al., 1978). And the lipophilic vacuoles were also found in mammals of the PCB toxicational experiment (Fishbein, 1974).

Histopathological of fish liver

A variety of histipathological changes have been found in the livers of many fish species exposed to different toxicants.

Effect of metals

The sublethal toxicity associated with exposure of adult lake whitefish (*Coregonus clupeaformis*) to diets containing 0, 10, 100 and 1000 μg Ni per g for 10, 31 and 104 days. Histological lesions in liver were showed areas of focal necrosis, altered bile ducts were observed and significant increases in lipid peroxide concentration (Ptashynski et al., 2002). In lake whitefish fed uranium-contaminated diets found abnormal architecture of hepatic plate, cell necrosis, hydropic swelling, foci area of liver parenchyma and bile duct epithelium was alteration (Cooley et al., 2000). Adult lake whitefish were fed arsenic on diets were observed nuclear, architectural and structural alteration, areas of inflammation and focal necrosis. Sloughing oh epithelium, dilation of vascular elements, inflammation, edema, fibrosis and increase width of the submucosa were some of the alterations observed in gallbladders (Pedlar et al., 2002). *Heteropneustes fossilis* were exposed to cadmium at 57 mg/L for 30 days investigated alteration of nucleus, fragmentation and vacuolation in the cytoplasm (Ghosh and Chakrabarti, 1993). Investigated the vital role of arsenic in rainybow trout at the early life stage of development. The histological analysis showed that the degeneration and inflammatory lesions and nonneoplastis proliferative lesions (Kotsanis and Iliopoulou-Greorgudak, 1999). An ecosystem contaminated with a variety of metal and organic chemicals that exposure to environmental contaminants from polluted wetlands typical of the Mississippi River Basin. The lesions occurred in three species of gar (Lepisosteus osseus, L. oculatus and *L. platostomus*) observed were inflammation and multifocal necrosis in the liver organ (William, 2000). Trout were fed a pellet diet for 42 days

contaminated with mercuric chloride to provide a mercury concentration of 10 g metal kg dry weight of food. Histological change was showed sloughing of the gut epithelium and food regurgitation occurred in the last 3 weeks of the study, but no mortalities were observed. (Handy and Penrice, 1993).

Effect of fungicides

Study effect of malachite green on the liver of rainybow trout (*Salmo gairdneri)* at concentration 6 ppm. Study under light microscope they found blood congestion in sinusoid, foci necrosis in many areas. And study under electron microscope that showed mitochondria damage, cristae swelling, cisternae of endoplasmic reticulum abnormal, and severe change of nucleus (Gerundo et al., 1991). Histopathological changes observed in the liver of rainybow trout (*Oncorhynchus mykiss)* contaminated to triphenyltinacetate found an irregular of hepatocytes, accumulation of fat vacuole and inflammatory areas (Sehwaiger et al., 1996)

Effect of herbicides

Chronological effects produced by 0.17 mg atrazine in the liver of juvenile grey mullets (*Liza* sp.) were investigated by means of light and electron microscopy. Fish primarily reacted (day 3 to 9) with a substantial increase in the size of lipid droplets (adaptive change) and lipofuscin granules. After 21 days of contamination, liver lipoid degeneration was observable. Such pathology indicates the transition to the final step of the stress process (exhaustion) as revealed by the increase in mortality rates. In mullets intoxicated for 11 days then returned to clean water for 18 days, reversal of the induced alterations and enhancement of the hepatic metabolism linked to detoxication mechanisms indicated hepatic recovery. These results provide evidence of ultrastructural perturbations in mugilid liver by a subacute concentration of atrazine (Biagianti-Risbourg and Bastid, 2000). Glyphosate herbicides were exposed on liver of carp (*Cyprinus carpio*) showed that blood congestion in few sinusoid and focal fibrosis (Neskovic et al., 1996).

Effect of water pollution

Khan et al. (1994) study effluent originating from pulp and paper mills have been reported to induce a variety of histological alteration in fish including the winter flounder, *Pleuronectus americanus*. These effect that focal vacuolation, pyknotic nucleus, fatty accumulation and macrophage aggregation in the liver. Moreover bile duct hyperplasia with increases wall thickness and granulomas (pericholangiolar fibrosis). The same experiment of Khan (1998) indicated that histological changes in the liver of feral winter flounder (*Pleuronectus americanus)* that were captured from the petroleum refinery in New foundland. Examination of tissue revealed in the liver basophilic staining hepatocytes, pericholangitis and macrophage aggregates with hemosiderin. Additionally, bile duct hyperplasia was also presented. Folmar et al. (1993) reported that the brown bullheads (*Ameiurus nebulosus)* collected from the Buffalo and Niagara Rivers in New York have a high incidence of liver cancer. These locations contain a variety of chlorinated pesticides, PCBs, dioxin, aromatic amines, polynuclear aromatic hydrocarbons and metal. And histological changes of crucial carp (*Carassius auratus)* liver that exposed to sediments from lake contaminated with dioxin and related compounds. These damages were found extreme enlargement of hepatocytes, condense and irregular cell nucleus, polynuclei, dispersed heterochromatin, enlargement of the nucleolus and degeneration of the nucleus (Wu et al., 1999).

Effect of insecticides

Adult female rainybow trout (*Oncorhynchus mykiss)* fed TCDD impregnated diet at 0, 1.8, 18 and 90 ng/Kg food for up to 320 days. This experiment showed less hepatocellular glycogen, more mitotic figures, greater anisokaryosis, anisocytosis, nuclear chromatin clumping and margination. Furthermore, found single cell necrosis and clear cytoplasmic vacuoles consistent with lipid (Gail et al*.,* 2000). Histological changes in the liver of *Tilapia mossambica* were observed after exposure to a sub lethal level (2.5 ppm) of monocrotophos. The changes determined after 2 days of exposure were characterized by necrosis and vacuolation of hepatocytes. Fatty degeneration was observed after 10 days of exposure. (Desai et al*.,* 1984). Jonsson and Toledo (1993)

reported that the acute toxicity of endosulfan to zebra fish (*Brachydanio rerio)* and yellow tetra (*Hyphessobrycon bifasciatus)*. The liver of both species found lipid accumulation, zonal necrosis, and mononuclear inflammatory infiltrates. In *tandanus tandanus* contaminated with endosulfan showed atrophy hepatocyted, pyknotic nucleus and fat droplet in cytoplasm. In addition found dilation of sinusoid rupture of membrane (Nowak, 1996). *Tilapia mossambica* exposure to fenvalerate caused histopathological changes. These effects showed foci necrosis, pyknotic nucleus and degeneration of cytoplasm (Radhaiah and Jayantha-Roa, 1992). Fenvalerate, a synthetic pyrethroid 3.45 mg/l was treated in the fish *Ctenopharyngodon idellus* decrease levels of glycogen and proteins in liver tissue were observe after contaminated for 8 days. Histophatological changes are observe the tissue damages like necrosis, vacuolar degeneration and dtrophy (Tilak and Yacobu, 2002). The biochemical tests were conducted to find out the relationship between liver glycogen and endosulfan toxicity on a cat fish *Heteropneustes fossilis* using 0.00075, 0.00050 and 0.000375 ppm concentration for 15, 30, 45 and 60 days of exposure periods. The quantity of liver glycogen showed decreasing trend as concentration to toxicant increase. The depletion in glycogen contents in greatly affiliated to cellular damage in hepatocytes (Rawat et al., 2002). The investigation was undertaken to evaluate the toxicity of dimecron (phosphamidon) an organophosphate pesticides used in agriculture. Long term exposure to sublethal cocentration 0.00068 ppm of dimecron on liver found major changes were hepatic lesions with necrosis pyknotic nuclei, vacuolation, damage blood vessel and accumuation of cytoplasmic granule in liver tissue (Sakthivel and Gaikwad, 2002).

CHAPTHER III

MATERIALS AND METHODS

3.1 Experimental animal

The experiments were performed with a species of freshwater fish, the Common Silver Barb *Puntius gonionotus* commonly known in local name as Pla Ta Pien. The fish collected from Klong 7 Pathumtani Province was referred as a natural group. The control group at the age of 1 month old was obtained from Mongkol Farm. The control group was then acclimated to the laboratory condition for a period of about 12 weeks in 325 L glass aquaria before experimentation to ensure that they were disease-free and recovered from transportation stress. The Common Silver Barb age of 4 month old, were exposed to endosulfan at concentration level of 0.06, 0.25, 0.50 and 1 ppb as the treated groups. Fish were fed with commercial pellet (CP company) food twice a day.

3.2 Chemicals

Chemical required for histological study and gas chromatography analysis are summarized in Appendix A.

3.3 Apparatus

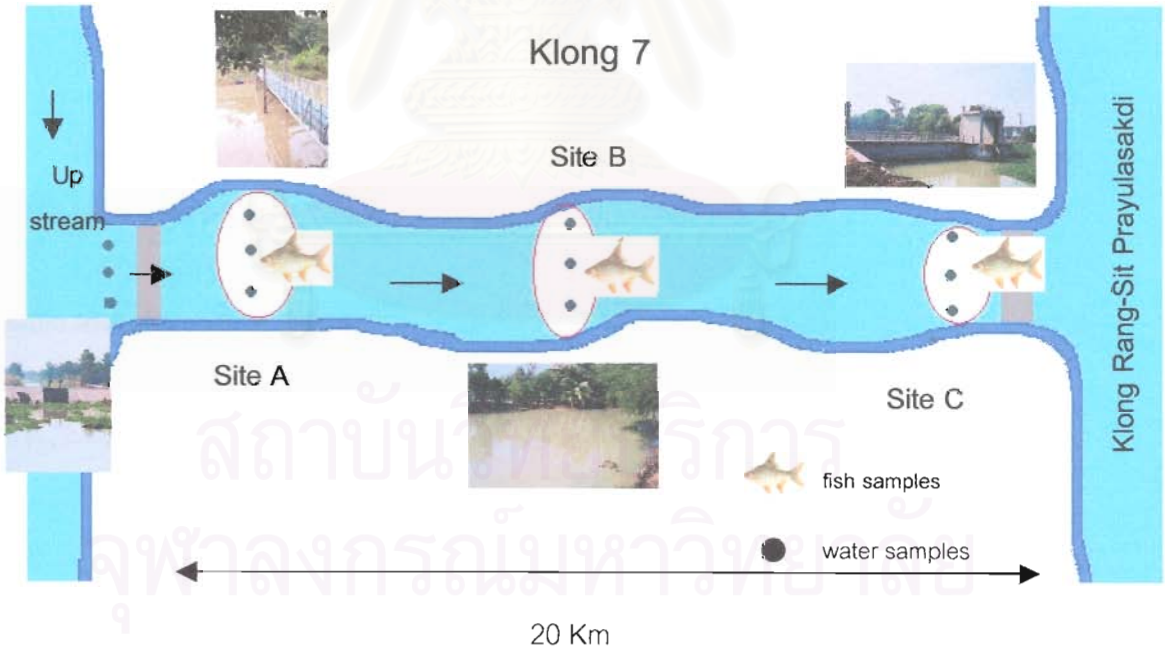
Apparatus and equipment required for fish culture, toxicity test, histological study and gas chromatography analysis are summarized in Appendix A.

3.4 Field study

The fish samples and water samples were collected every month during March to December 2004 from Klong 7 in two season, rainy season (April to October) and dry season (November to March).

1. Fish samples were collected from three sites along 20 kilometers of Klong 7 sub-canal (Figure 3.1).
2. Water samples were collected from four sites, up steam, site A, B and C respectively (Figure 3.1). The water sample was individually collected using 1-liter amber bottle. There were three replicates of the water sample at each site. The water samples were maintained at 0 to 4°C from the time of collection until extraction.
3. Dissolved oxygen, pH, specific conductivity and water temperature were measured *in situ* at each site during the sampling period.

Figure 3.1 Map of study sites at Klong 7, Pathum Thani Province



3.5 Laboratory study

A. Histological study

1. Fish samples were individually measured for the total length and body weight (wet weight).
2. The liver of each fish was dissected from the body and characteristics were observed (size, shape and color). Then the liver weight (wet weight) was measured. The relative liver weight index, %RL or hepatosomatic index) was computed by dividing the fish liver weight by its body weight.
3. The liver was fixed in 10% buffered formalin for paraffin technique and frozen technique.
4. The relative liver weight index was separately performed in each season for statistical testing. The analysis of differences among control, Klong 7 and endosulfan treated groups were performed by one-way ANOVA, Post Hoc tests with Duncan's new multiple range test at 95 % confidence interval.

There are two categories of light microscope study techniques; the standard paraffin technique and the frozen technique.

Paraffin technique (Gurr, 1963)

This technique was performed as follows;

1. The fish liver was cut into small pieces of about 0.5 – 1 cm^3 and fixed in 10% buffered formalin for 24 hours at room temperature.
2. The liver tissues were dehydrated in the series of 70%, 90%, 95% of ethanol and n-butanol respectively; then embedded in paraplast.
3. The paraffin blocks were cut to 5 μm thickness by the rotary microtome.
4. The ribbons of these sections were attached to the slide by egg albumin and dried at 40^{o}C on warm plate.

5. The sections were deparaffined with xylene and hydrated in the series of n-butanol, 95%, 90% and 70% of ethanol, respectively before staining by heamatoxylin and eosin (H&E).
6. The slides with stained tissue were mounted with permount and then examined under the light microscope.

Frozen technique

Frozen technique was used for the study on the histochemistry and this technique was performed as follows;

1. The liver was cut into a small piece of about 3 mm^3 and then rapidly picked up in foil molds and filled in with the frozen medium (tissuetek).
2. The molds were immediately cooled down at -20^{o}C
3. The frozen tissues were cut at 5 μm thickness by the freezing microtome
4. The sections were attached to the slides and air dried before staining.

Two staining techniques were used in this study for the histochemical investigation.

Oil red O staining

The Oil red O staining techniques were used for the study on the lipid composition of the fish liver (Culling, 1963) and this technique was performed as follows;

1. The sections were immersed in 70% ethanol before immersed in Oil red O.
2. After rinsing in distilled water, the sections were immersed in Heamatoxylin.
3. The sections were washed in running water until blue and were mounted with glycerin jelly and then examined under the light microscope.

The Periodic acid / Schiff (PAS) technique

The Periodic acid / Schiff (PAS) technique was used for the study on the value of glycogen composition (Gurr, 1963) and this technique was performed as follows;

1. The sections were immersed in Periodic acid, washed in distilled water, stained in Schiff reagent, stained in Schiff reagent, washed in distilled water, dehydrated with 70%, 90% and 95% ethanol and cleared in xylene.
2. Then they were mounted with permount and examined under the light microscope.

Endosulfan treated group

The test was conducted in 12-L glass jars containing 10 L of different concentrations of the endosulfan solution. Four jars were filled with the treated solution at 0.06, 0.25, 0.5 and 1 ppb while one jar was filled with holding water and left as control. All experimental conditions had two replicates and 3 fish, age 4 month old, were used for each jar. The fish of undetermined sex were randomized from the holding aquaria and assigned to the experimental jars. Mortality was observed and recorded every 24 hours until the end of the experiment (8 days). Fish liver was fixed in 10% buffered formalin.

After that, the liver tissue was processed by the following paraffin standard method and permanent slide staining by heamatoxylin/Eosin. Sections from he section of frozen technique were stained by PAS (Gurr, 1963) and Oil red O (Culling, 1963).

For the semi-quantitative study, all slides were studied under the light microscope and the semi-quantity of specific lesion of liver was defined to

-	=	not present
+	=	mild degree of lesion
++	=	moderate degree of lesion
+++	=	strong degree of lesion
++++	**=**	extreme degree of lesion

B. Gas chromatography Analysis

Gas Chromatography (GC) method for determination of certain organophosphate pesticides in wastewater is comparable to USEPA method 8270.

Water sample extracts were analyzed using Hewlett Packard HP-6890N gas chromatographs that have autosample injector in 7683 Series and gas chromatography Flame Photometric Detector (FPD).

Calibration and standardization

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

External standard calibration procedures

The ranges of the calibration standards were prepared at 1, 2, 5, 8 and 10 mg/l (ppm) concentration levels. Analysis of pesticides standards for the calibration curve was conducted FPD. The order and peaks of organophosphate pesticides (OPs) shown in the chromatogram. The area of each peak was measured and plotted against the relationship between them concentration in order to see the linear.

Extraction (modified from USEPA method 8270)

Liquid Liquid Extraction (LLE) by using separatory funnel

1. Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Add preservative to blanks and QC checks standards.
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 hexane: dichloromethane 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. The solvent extract was collected in Turbo Vap tube by filter solvent layer through a bed of granular anhydrous sodium sulfate.

3. The 1: 1 hexane: dichloromethane solvent 50 ml was transferred to separatory funnel and repeat the extraction procedure a second time, combining the extracts in Turbo Vap tube by filter solvent layer through a bed of granular anhydrous sodium sulfate.
4. Repeat in step 3

Extraction concentration

The extracted sample was transfer into the Turbo Vap tube and concentrated into the final volume 2 ml by constant temperature 40°C. Afterward, the concentrated sample was kept in TFE-fluorocarbon screw-cap vial, and then it was stored in the refrigerator at -5°C before analysis by GC/FPD.

Gas Chromatography (GC)

Gas Chromatography (GC) was used to determine the concentration of organophosphate residues.

1. Gas Chromatography were performed with capillary columns, HP 6890 gas chromatography and autosample injector 7683 series of Hewlett Packard. The GC column was used HP-5 (5 % diphenyl, 95 % dimethyl polysiloxane) ID 0.32 mm, Film 0.25 μm and Using Flame Photometric Detector (FPD) and analysis by Software Chemstation of Agilent Technologies.
2. Instruments condition were
   - Injection temperature 210°C
   - FPD detector temperature 250°C
   - Split less: Purge flow to split vent 60 ml / min 0.75 min
   - Make up gas for detector was N_2 with an initial column temperature of 100°C for 2 min, a program rate of 10°C / min to 280°C hold 5 min to 300°C hold 4 min. Autosample was injected volume 1 μl. Total run time is 29 minutes.

Identification analysis, Data analysis and calculations

One microlitre was injected into GC. If an extracted sample contains organophosphate pesticides (OPs), it can be analyzed the quality and quantity of each OPs by the comparison of the retention time and peak area with OPs standard. The concentration (C) in the sample can be calculated from this equation below;

$$\text{Concentration of Ops in water (mg/l)} = \frac{CAa}{VB}$$

where:

C = the concentration of Ops in standard solution (mg/l)

A = peak area of Ops in sample

B = peak area of Ops in standard

V = final volume of sample (ml)

A = volume of water sample (ml)

Percent recovery

One way to determine accuracy or the efficiency of extraction is to spike test portions with the analyte at various concentrations, then extract the fortified test portions and measure the analyte concentration.

1. The analysis of percent recovery, sets matrix standard two concentrate levels of reagent water spike, sets of seven replicates of each concentrates and run according extract method and analyzed by gas chromatograph.
2. Using results compute the average percent recovery of matrix standard. The recovery of matrix standard shall be with in the limits of 70 to 120 percent.

$$\text{Recovery (\%)} = (C1 - C2) / C3 \times 100$$

Where C1 = concentration determined in fortified sample

C2 = concentration determined in unfortified sample

C3 = concentration of fortification

Limit of Detection (LOD)

Where measurements are made at low analyte, it is important to know what is the lowest concentration of the analyte that can be confidently detected by the method. The importance in determining this, and the problems associated with it, arise from the fact that the probability of detection does not suddenly change from zero to unity as some threshold is crossed.

LOD = 3 S/N (signal/noise)

Limit of Quantitation (LOQ)

The LOQ is strictly the lowest concentration of analyte that can be determined with an acceptable level of repeatability precision and trueness. It is also defined by various conventions to be the analyte concentration corresponding to the sample blank value plus standard deviations of the blank. LOQ is an indicative value and should not normally be used in decision making.

LOQ = 10 S/N (signal/noise)

Relative standard deviation (%RSD)

The Field replications, three replicates water samples were collected from each site of Klong 7 Pathum Thani Province. The amounts of organophosphate insecticides residues concentrations were determined, which were used for precise then %RSD (Relative standard deviation) sample replication.

This value assess to precision of standard method.

%RSD = SD/ Mean x 100

Quality control

The major parameters that should be known and kept in control in order to generate quality analytical data, the precision 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 various types of blanks ensures that each step of the analysis is free of significant contamination. Finally, extraction steps must be check for extraction efficiency. A matrix spike or standard reference material is used to ensure that the analyte is being properly extracted. 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 blanks as test of continued performance and analysis of spiked samples to assess accuracy.

Blank: Reagent water reference sample blank, solvent blank and system blank were analyzed to demonstrate freedom from contaminates.

Field replication: to assess precision, property used, this gives the analyst more information on the underlying statistics behind a particular measurement. Experiments involving replicate analysis should be designed to take into account all of the variations in operational conditions that can be expected during routine used of method. The aim should be to determine typical variability and not minimum variability.

CHARPTER IV

RESULTS

Gross anatomy of the Common Silver Barb liver

Fish liver was found lining in the abdominal cavity above the pelvic fin. Large liver was not clearly separated into lobe and connected with the digestive tract. The upper part of the liver was large and slightly smaller in the lower part. In the control group liver, the color was yellow or yellow to brown.

The liver of Klong 7 fish and endosulfan treated fish was light yellow with white spots that distributed through out the liver tissue. The liver tissue was rather soft and the size was often smaller than the liver tissue of control fish.

Relative liver weight index (%RL)

The means of length, body weight and liver weight of the control group and Klong 7 group in dry season and rainy season at each site were recorded and calculated for %RL. The fish at Klong 7 were 3-4 times heavier in weight than the fish in the control and endosulfan groups. The means of the body weight were 7.460 $\pm$ 1.113, 27.860 $\pm$ 2.868 and 21.540 $\pm$ 2.858 g in control group, Klong 7 dry season and Klong 7 rainy season group, respectively. The means of the body weight were 3.825 $\pm$ 0.208, 3.633 $\pm$ 0.329, 3.978 $\pm$ 0.524 and 4.090 $\pm$ 0.290 g in endosulfan treated group at the concentration level of 0.06, 0.25, 0.50 and 1 ppb, respectively (Table 4.1).

From statistical analysis by one-way ANOVA and Duncan's Multiple Range Test, the %RL of the Klong 7 group was significantly lower than the control group ($p \leq 0.05$). There were 2.010 $\pm$ 0.127, 1.434 $\pm$ 0.113 and 1.350 $\pm$ 0.077 in control, Klong 7 dry season and Klong 7 rainy season group respectively. However, %RL between Klong 7 dry season and Klong 7 rainy season were not significantly different. %RL of endosulfan treated fish were 1.716 $\pm$ 0.360, 1.666 $\pm$ 0.215, 1.640 $\pm$ 0.208 and 1.758 $\pm$ 0.198 at the concentration level of 0.06 ppb, 0.25 ppb, 0.50 ppb and 1 ppb, respectively. From the mean comparison of %RL among groups, it was found that all endosulfan treated

groups showed significant decrease in %RL compared to the control group and there was no significant difference among endosulfan treated groups. Comparisons between Klong 7 groups and Endosulfan treated groups indicated that % RL of Klong 7 groups were significantly lower (Table 4.1).

Table 4.1 Means of length, body weight and liver weight of control fish, Klong 7 fish in dry and rainy season and endosulfan exposure fish.

Group	Length (cm) $\bar{X} \pm SE$	Body weight (g) $\bar{X} \pm SE$	Liver weight (g) $\bar{X} \pm SE$	% RL $\pm$ SE
Control (N=34)	8.060 $\pm$ 0.329	7.460 $\pm$ 1.113	0.150 $\pm$ 0.019	2.010 $\pm$ 0.127^{a}
Klong 7 Dry season (N=70)	11.500 $\pm$ 0.434	27.860 $\pm$ 2.868	0.399 $\pm$ 0.014	1.434 $\pm$ 0.113 *^{b}
Klong 7 Rainy season (N=44)	10.590 $\pm$ 0.398	21.540 $\pm$ 2.858	0.290 $\pm$ 0.013	1.350 $\pm$ 0.077 *^{b}
Endosulfan treated 0.06 ppb (N=6)	7.000 $\pm$ 0.258	3.825 $\pm$ 0.208	0.065 $\pm$ 0.011	1.716 $\pm$ 0.360 *^{c}
Endosulfan treated 0.25 ppb (N=6)	6.916 $\pm$ 0.200	3.633 $\pm$ 0.329	0.060 $\pm$ 0.008	1.666 $\pm$ 0.215 *^{c}
Endosulfan treated 0.50 ppb (N=5)	7.100 $\pm$ 0.331	3.978 $\pm$ 0.524	0.065 $\pm$ 0.008	1.640 $\pm$ 0.208 *^{c}
Endosulfan treated 1 ppb (N=6)	6.830 $\pm$ 0.100	4.090 $\pm$ 0.290	0.071 $\pm$ 0.010	1.758 $\pm$ 0.198 *^{c}

* = significant difference ($p \leq 0.05$)

subscript = significant difference ($p \leq 0.05$)

Histological alteration of fish liver

1. Control liver

The polygonal hepatocytes in normal liver of the Common Silver Barb are arranged between the sinusoids as a mesh-like network of branching cords. The sinusoids, central vein and other blood vessels are dispensed between the hepatic parenchyma (Plate 1 Figure A). The normal hepatocytes have nucleus with disperse chromatin that concentrated closely to the nuclear membrane and contain single nucleolus. Hepatocyte nucleus is finding at the central of cell or basal side. The cytoplasm of hepatocytes often contains granules, lipid droplets and vacuoles (Plate 1 Figure B and C). Hepatic tissues compose of hepatic parenchyma, bile duct and pancreatic tissue (Plate 1 Figure E). The capsule lined with single layer of simple squamous cell (Plate 1 Figure F).

2. Klong 7 liver

2.1 Klong 7 in dry season liver

The liver of Common Silver Barb in dry season at Klong 7 showed the sign of cellular injury with consist of hydropic swelling hepatocyte in the area close to blood vessel, ruptured of endothelial of blood vessels, necrotic red blood cell in blood vessel (Plate 2 Figure A). Moderate hyalin droplets accumulation and lipid droplets accumulation were found (Plate 2 Figure B). Central vein showed the blood congestion, endothelial layer rupture and thickness (Plate 2 Figure C). Liver of many case displayed foci necrosis and diffuse necrosis was found in some case, the spot of necrosis area was observed at sinusoid, blood vessel and the margin of the liver parenchyma (Plate 2 Figure D - F). Pyknotic and karyolytic nucleus (Plate 3 Figure A - E) and sloughing of liver capsule layer were found (Plate 3 Figure F). In the area of inflammation, there were some lymphocytes, granulocytes and macrophage infiltration in liver parenchyma especially at the area near the blood vessel (Plate 4 Figure A). In addition, dilatation of sinusoid (Plate 4 Figure B), abnormal shape of red blood cell (Plate 4 Figure C), eosinophilic cells and eosinophilic area were noticed. Slightly fibrosis (fibroplasias) was also detected in some case as a repair tissue that response to cell necrosis (Plate 4

Figure D). In addition, group of necrotic cell packing with fibrous were found near the endothelial layer of blood vessels and in the liver parenchyma (Plate 4 Figure E, F). Moreover group of abnormal cells in blood vessel (Plate 5 Figure A), and lipid accumulation and fusion into large area of fatty degeneration were seen (Plate 5 Figure B).

Not only the hepatocytes injury but also the damage of bile duct and pancreatic tissue and cell debris in duct lumen was reported. Necrosis of pancreatic cell and pancreatic tissue was shrinkage (Plate 5 Figure C), inflammation of the pancreas was indicated by infiltration of granulocytes and macrophages (Plate 5 Figure D). Injury of pancreatic cell was noticed as the lost of its zymogen granules (Plate 5 Figure E and F). (Table 4.2)

2.2 Klong 7 in rainy season liver

The liver of the Klong 7 fish showed cellular injury. The liver alteration consists of hyalin droplet in cytoplasm of hepatocytes, blood congestion in sinusoid and central vein (Plate 6 Figure A) and dilatation. Foci necrosis and diffuse necrosis were found (Plate 6 Figure B). The slight accumulation of lipid vacuoles in cytoplasm of hepatocytes was observed in this season (Plate 6 Figure C and D). At the liver area around blood vessels, hepatocytes were seen hydropic swelling and several liver showed revealed foci necrosis. The most severe lesion liver showed the inflammation that defined by the infiltration of lymphocytes, granulocytes and pigmented macrophage into liver parenchyma and often in the area around blood vessels (Plate 6 Figure E and F). There was the sloughing of capsular epithelium of liver, endothelial of central vein and bile duct epithelium (Plate 7 Figure A - C). Endothelial cells of central vein were thicker than endothelium cell of control group (Plate 7 Figure D). Abnormal form of red blood cell and necrosis red blood cell were seen near endothelial layer of central vein, and in the liver parenchyma (Plate 7 Figure E). Some area, eosinophilic cells and eosinophilic area of necrosis cell were noticed (Plate 7 Figure F). Additionally, nucleus of hepatocytes become shrinkage and degenerate as it was called pyknotic nucleus and karyolysis, respectively (Plate 8 Figure A - F). Mass of blood clots was found in liver

parenchyma of one case (Plate 9 Figure A) and Abnormal form of hepatic cell (Plate 9 Figure A).

Bile duct and pancreas showed cell injury. The bile duct had abnormal architecture with the twist of its lumen. Distort pancreas and foci necrosis change the structure of pancreatic tissue. Moreover, it was found the blood congestion in blood vessels of pancreas (Plate 9 Figure C). Pancreatic cells were necrosis and lost of zymogen granules (plate 9 Figure D - F). (Table 4.2)

3. Endosulfan treated liver

Klong 7 agricultural area, there are many insecticide residues contaminated in water. Endosulfan is one of them that often contaminated in this area, therefore the endosulfan toxicity test group was set up by using 0.06 – 1 ppb concentration levels for sublethal toxicity of endosulfan on Common Silver Barb. Four groups of fish were exposed to 0.06, 0.25, 0.50 and 1 ppb of endosulfan for 8 days. Histological change of liver was studied under the light microscopy (Table 4.3).

3.1 liver of 0.06 ppb of endosulfan treated fish

The treated liver showed sign of cellular injury, that consist of a slightly blood congestion in vessels and sinusoids (Plate 12 Figure B). Thickening and rupture of endothelial lining, lipid vacuole accumulate in cytoplasm of hepatocytes and cell necrosis (Plate 12 Figure C). The pancreas was damaged and without zymogen granules and bile duct shrinkage (Plate 12 Figure D), some liver showed foci necrosis and macrophages infiltration (Plate 12 Figure E). Most of the capsule of liver was still have simple squamous epithelium as normal liver (Plate 12 Figure F).

3.2 liver of 0.25 ppb of endosulfan treated fish

The treated liver showed the sloughing endothelium in central vein and blood congestion and sinusoid dilation. Hyalin deposition in cytoplasm of hepatocytes and macrophage infiltration were noticed (Plate 13 Figure B). Pyknotic nucleus, karyolysis and foci necrosis around central vein were detected (Plate 13 Figure C).

Pancreas and bile duct were distorted, pancreatic cell necrosis was seen in many liver (Plate 13 Figure D). Eosinophilic cytoplasm of hepatocytes and dead red blood cell were seen in necrotic area (Plate 13 Figure E). The inflame pancreas also showed the infiltration of macrophage and fragments of red blood cells (Plate 13 Figure F).

3.3 liver of 0.50 ppb of endosulfan treated fish

The liver of exposed fish showed moderately cellular injury. Blood congestion in sinusoid and central vein, red blood cell without cytoplasm, endothelial rupture of blood vessels were showed (Plate 14 Figure B). Moreover, there were hepatic cell swelling and foci necrosis around blood vessels, lipid droplet accumulation and endothelium separation (Plate 14 Figure C). Red blood cell swelling, pancreatic cell necrosis, and blood congestion in blood vessel of pancreas (Plate 14 Figure D) and sinusoid dilation (Plate 14 Figure E). Pancreatic cell found necrosis without zymogen granules were noticed (Plate 14 Figure F).

3.4 liver of 1 ppb of endosulfan treated fish

Most of the livers were damaged, the consisted of thickening and endothelial rupture in sinusoid and central vein, Encapsulated of necrotic cells aggregated in blood vessel, foci necrosis and cell edema (Plate 15 Figure B). Diffuse necrosis was observed through out the liver parenchyma and also the macrophage infiltration (Plate 15 Figure C). Sloughing of hepatic capsule, pyknotic nucleus and foci necrosis around the central vein and blood congestion were seen (Plate 15 Figure D, E). The bile duct shrinkage and lumen of duct was narrow (Plate 15Figure F).

Comparison the degree of liver alteration of Klong 7 fish between three sites study

In dry season, degree of cell injury in each site is not different. Most of the liver was irreversible injury that eosinophilic cell, pyknotic nucleus and karyolysis. Reversible damage level slightly observe example cell swelling, inflammatory of tissue that found macrophage and white blood cell infiltration. Comparisons damaged of the Klong 7 fish liver change in each site in dry season (Appendix C).

In rainy season, histopathological alteration is not different between sites. Majority of cell damage is irreversible level found cell die both foci and diffuse necrosis some case found hepatic plate abnormal. Moreover observe fat accumulation in cytoplasm of hepatocytes and fusion of lipid becoming widen lipid deposit area that effect cell necrosis. Comparison degree of lesion liver alteration of Klong 7 fish between site studies in rainy season (Appendix C).

Comparison the degree of liver alteration of Klong 7 fish between seasons.

The liver changes of the Klong 7 fish between dry and rainy season were slightly different. From the comparison the events of reversible cell injury and the irreversible cell injury, liver of dry season fish showed greater alteration than the rainy season fish. Since dry season fish liver had hyalin droplets, which was the protein, denature deposition in cytoplasm of hepatic cell, eosinophilic area, pyknotic nucleus, karyolysis and cell necrosis more than the liver of rainy season fish. Moreover it was found hepatic plate disarrangement too (Plate 10 Figure B and C). In rainy season lipid droplets accumulated in cytoplasm of hepatocytes and lipid droplets fusion which according to the necrosis of hepatic cells (Plate 10 Figure D and E). And the other lesions both seasons are similarity discuss violent injury level (Table 4.2).

Comparison the degree of liver alteration of Klong 7 fish between sizes of fish

The body size of Klong 7 fish in this experiment classified to 2 body size large size indicate that body weight more than 40 g and body length more than about 15 cm and small size is body weight less than 40 g. In dry season, hepatic cell damage in large body size and small body size of Klong 7 fish were not different. Both dry and rainy season showed irreversible hepatocytes injury that found foci and diffuse necrosis and some case found both foci and diffuse necrosis in liver tissue. Moreover reversible damage sinusoid dilates, cell swelling and blood congestion were seen (APPENDIX C).

Table 4.2 The degree of lesion of liver alterations of Klong 7 fish between seasons

	Control (N=15)	Dry season (N=32)	Rainy season (N=32)
Hyalin droplet accumulation	-	++	+
Lipid droplet accumulation	++	+	++
Foci necrosis	-	+++	++
Diffuse necrosis	-	++	+
Foci and diffuse necrosis	-	+	++
Sinusoid dilate	-	+++	+
Blood congestion	-	++++	++
Endothelial damage	-	++++	++++
White blood cell infiltration	-	++	++
Pyknotic	-	+++	++
Karyolysis	-	++	++

N	number of fish
-	Not present
+	Mild degree of lesion
++	Moderately degree of lesion
+++	Strongly degree of lesion
++++	Extremely degree of lesion

Table 4.3 The degree of lesion of liver alterations of endosulfan treated liver

	Control	Endosulfan treated liver			
		0.06 ppb	0.25 ppb	0.5 ppb	1 ppb
Hyalin droplet accumulation	-	++	++	++	++
Lipid droplet accumulation	++	++	++	+	-
Foci necrosis	-	+	+	++	++
Diffuse necrosis	-	-	-	+	+
Foci and diffuse necrosis	-	-	-	+	++
Sinusoid dilate	-	+	++	++	++
Blood congestion	-	++	++	+	++
Endothelial damage	-	+	++	+	++
White blood cell infiltration	-	-	+	-	+
Pyknotic	-	+	+	++	++
Karyolysis	-	+	++	+++	+++

-	Not present
+	Mild degree of lesion
++	Moderately degree of lesion
+++	Strongly degree of lesion
++++	Extremely degree of lesion

Histochemical alteration of fish liver

1. Control liver

From histochemistry study, the Oil red O staining technique shows positive Oil red O staining of normal control liver with a small size lipid droplet that distributed through out the liver tissue (Plate 3 Figure A, C and E). The PAS staining technique for stain glycogen of the control liver shows positive PAS staining, the color was pink (Plate 3 Figure B, D and F).

2. Klong 7 liver

2.1 Klong 7 in dry season liver

Histochemistry study of Klong 7 fish liver in dry season were stained Oil red O technique for lipid study. Liver parenchyma showed positive stain of large size lipid droplets that larger than the control liver and have fat droplets less number of droplets than control liver (Plate 11 Figure C).

The PAS staining technique for staining glycogen showed is positive PAS staining that less density than control liver (Plate 11 Figure D).

2.2 Klong 7 in rainy season liver

Histochemistry study of fish liver at Klong 7 in rainy season, the Oil red O staining technique for lipid staining showed positive stain with the moderately size of lipid droplets in liver which same size with the control liver the number of fat droplets were found lesser than the control liver (Plate 11 E)

The PAS staining technique for glycogen stain showed the positive PAS staining the Klong 7 rainy season liver showed lesser glycogen deposition than the control liver (Plate 11 Figure F). And not detect difference glycogen deposition between dry and rainy seasons (Plate 11 Figure D and F).

3. Endosulfan treated liver

Histochemistry study of the liver of the Common Silver Barb was exposed to endosulfan.

The Oil red O staining technique for lipid staining showed a positive stain of small lipid droplet accumulation in cytoplasm. It was less density than the control liver when compared between control liver and each concentration level of treated liver. At the highest concentration level at 1 ppb, the treated liver showed the least density of lipid droplets accumulation (Plate 16 Figure A – F).

The PAS staining technique for glycogen staining showed the PAS positive. The endosulfan treated liver accumulated glycogen more than the control liver and the amount of glycogen deposition depended on the concentration level of exposure (Plate 17 Figure A – F).

Table 4.4 The degree of histochemical alterations on liver of control fish, Klong 7 fish and endosulfan treated fish.

	Control	Rainy season	Dry season	Endosulfan			
				0.06 ppb	0.25 ppb	0.50 ppb	1 ppb
Oil red O staining	++	++	++	+	+	+	-
PAS staining	++	+	+	++	+++	+++	++++

-	Not histochemical alterations
+	Mild histochemical alterations
++	Moderately histochemical alterations
+++	Strongly histochemical alterations
++++	Extremely histochemical alterations

Plate 1

Control fish liver

(H&E staining)

Figure A Photomicrograph shows the normal liver comprises of hepatic plate, the polygonal hepatocytes (H) are arranged between sinusoid (S) that connects with central vein (CV) and shows lipid droplets accumulation in cytoplasm of hepatocytes (*).

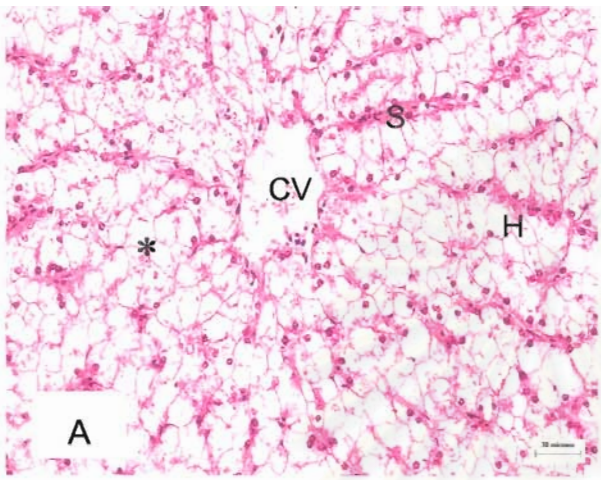
Figure B Photomicrograph shows round concentric nucleus of hepatocytes (→) with a single nucleolus (>).

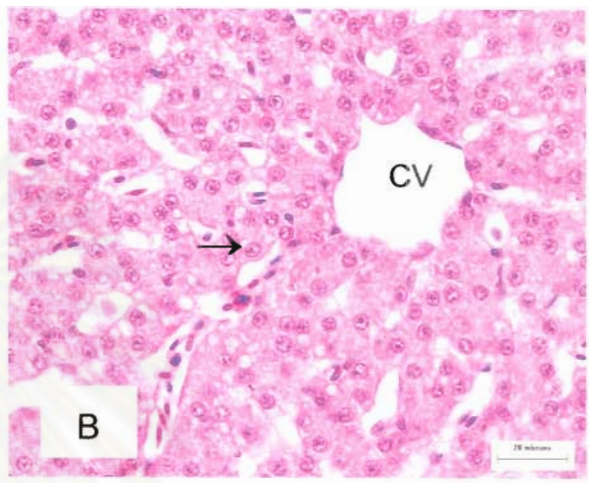
Figure C High magnification of liver photomicrograph shows hepatic cell with round concentric nucleus and a single nucleolus (→).

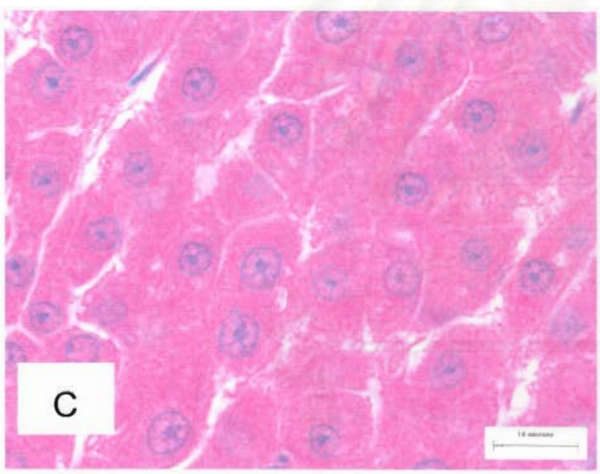
Figure D Photomicrograph of capsule shows a single layer of simple squamous cell lining (→).

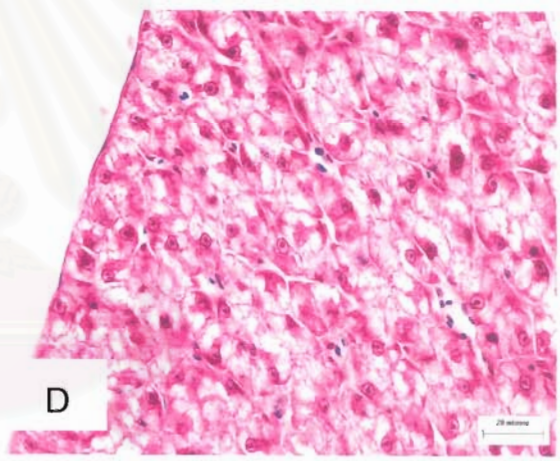
Figure E Photomicrograph showing bile duct (BD), pancreas (P), sinusoid and blood vessels that dispense between the hepatic parenchyma (→).

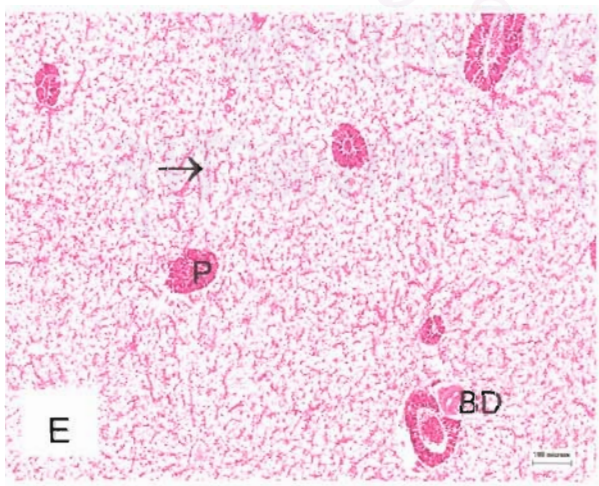
Figure F Photomicrograph of liver and pancreas shows zymogen granule in pancreatic cell (*) and lipid vacuoles (⟹) in cytoplasm of hepatic cell.


S
CV
H
*
A


CV
B


C


D


P
BD
E

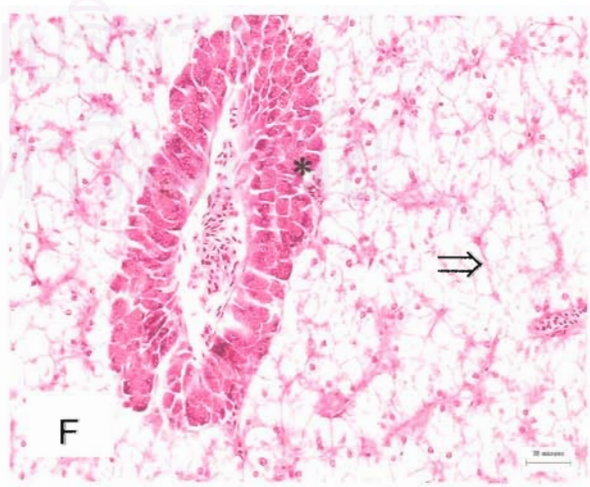

*
F

Plate 2

Liver of Klong 7 in dry season fish

(H&E staining)

Figure A Photomicrograph of the liver shows the rupture of endothelium of blood vessels (→) and cell debris in blood vessels (*).

Figure B Photomicrograph of the liver shows hyalin droplets accumulation in cytoplasm (→), cell swelling (>) around the central vein (CV) and foci necrosis (*).

Figure C Photomicrograph of the liver shows the thickening of endothelium of blood vessel (BV), the rupture of endothelium (→) and encapsulated necrotic cell (>).

Figure D Photomicrograph of the liver shows edema cell (→), fatty degeneration (L) and cell necrosis (*).

Figure E Photomicrograph of the liver shows diffuse necrosis at margin area of liver (*), hepatic plate disarrangement and the rupture of capsule (→).

Figure F Photomicrograph of the liver shows eosinophilic area of necrotic cell (*).

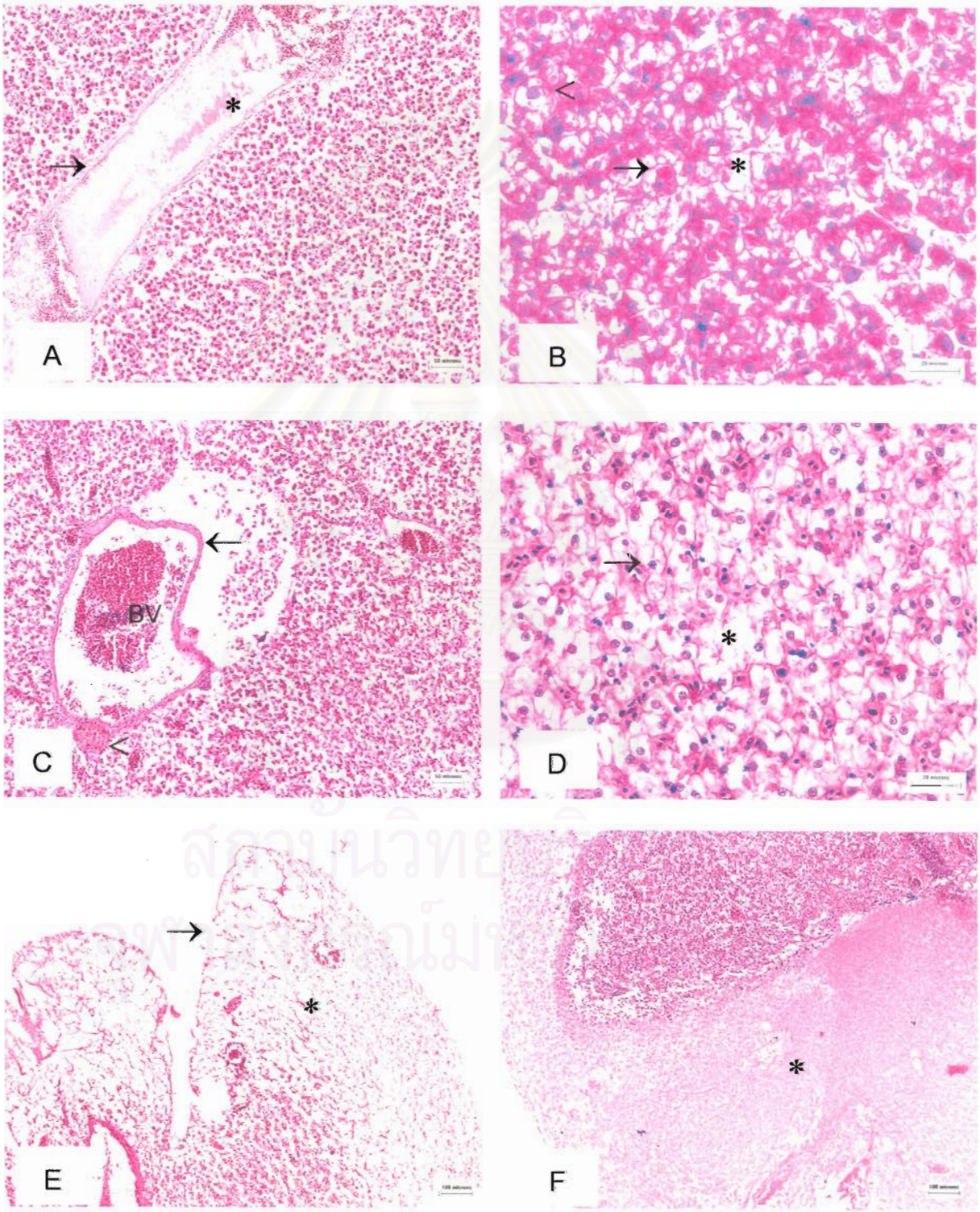

A
B
C
BV
D
E
F

Plate 3

Liver of Klong 7 in dry season fish

(H&E staining)

Figure A Photomicrograph of the liver showing pyknotic nucleus of hepatocytes at the area around central vein (→) and karyolysis (*).

Figure B Photomicrograph of the liver showing karyolysis (→), dilated sinusoid, blood congestion (>) and pyknotic nucleus (*).

Figure C Photomicrograph of the liver showing cell necrosis, karyolysis of hepatocytes (→).

Figure D Photomicrograph of the liver showing pyknotic nucleus (→), cell necrosis (*).

Figure E Photomicrograph of the liver shows cell lysis (*) area, the spot of necrosis area with pyknotic nucleus (→).

Figure F Photomicrograph of the liver showing cell necrosis (*) in large area and the rupture of liver capsule (→).

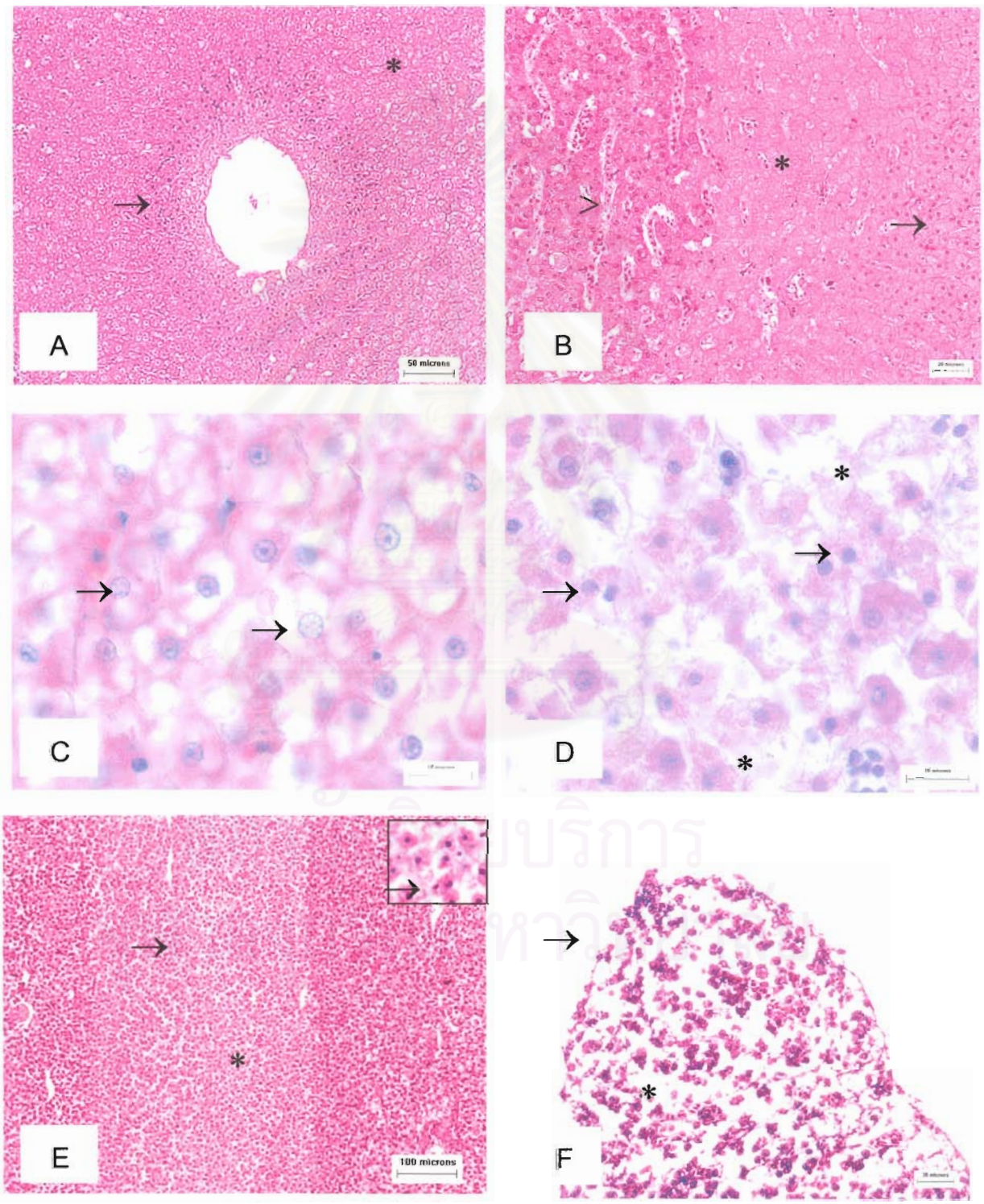

A
50 microns
B
C
D
E
100 microns
F

Plate 4

Liver of Klong 7 in dry season fish

(H&E staining)

Figure A Photomicrograph of the liver shows inflammation of liver tissue with the infiltration of granulocytes (→), macrophage (>), pyknotic nucleus (⇒) and cell necrosis (>>).

Figure B Photomicrograph of the liver shows sinusoid dilation (→) and cellular debris (*) in central vein.

Figure C Photomicrograph of the liver shows swollen red blood cell in blood vessel (→), lymphocytes infiltration (>), fibroplasias (*) in blood vessels and necrotic cell in blood vessel (⇒).

Figure D Photomicrograph of the liver shows eosinophilic area (*) and fibroplasias (⇒).

Figure E Photomicrograph of the liver shows encapsulated of aggregated necrotic cells layer of blood vessel, (*) under the endothelium of blood vessels, sloughing of endothelium (→) and cell necrosis around blood vessel (⇒).

Figure F Photomicrograph of the liver shows encapsulated of aggregated necrotic cells (*) in liver parenchyma, cell necrosis (⇒) and pyknotic cell (>).

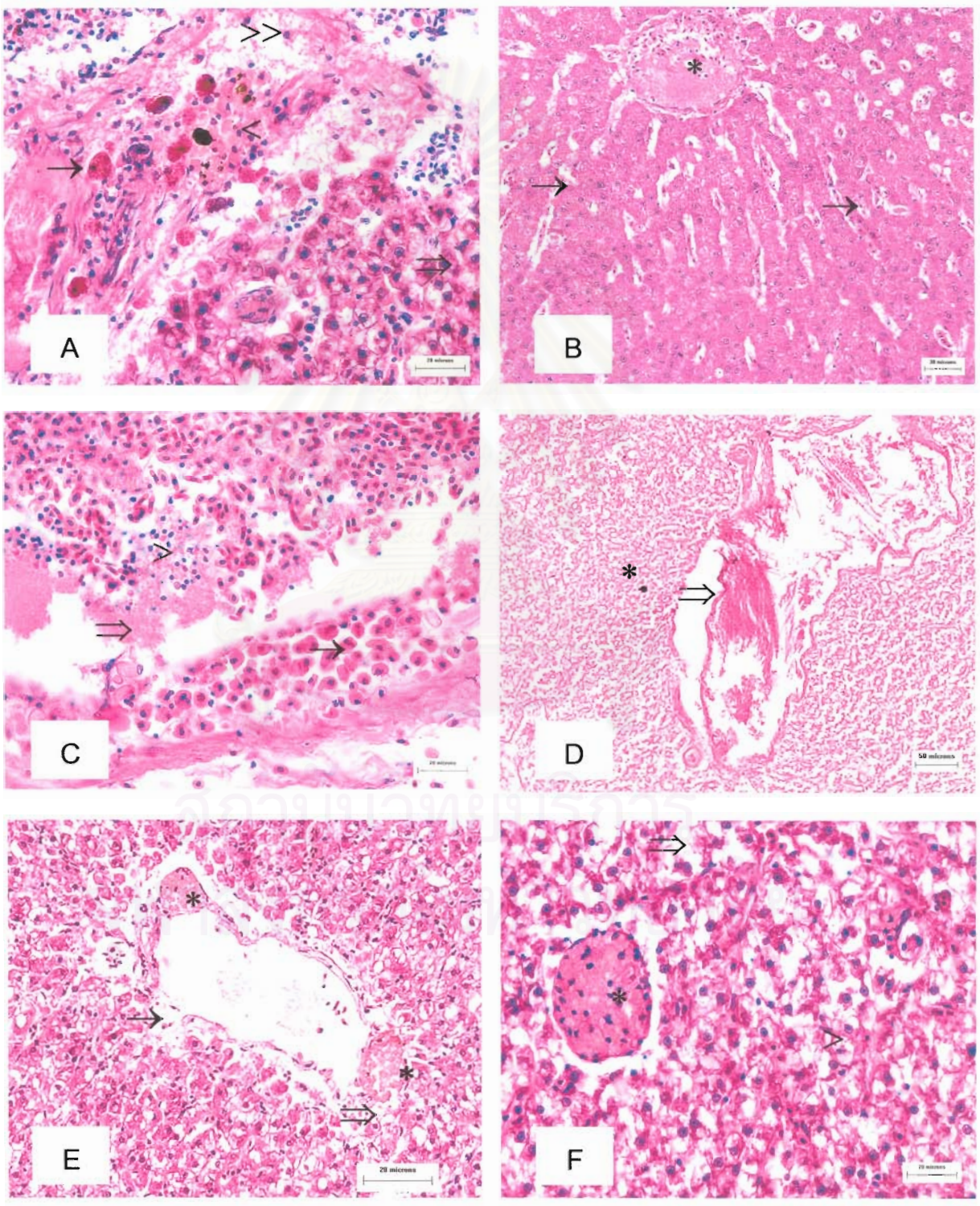

A
B
C
D
E
F

Plate 5

Liver of Klong 7 in dry season fish

(H&E staining)

Figure A Photomicrograph of the liver shows cell dead with capsule (*).

Figure B Photomicrograph of the liver shows lipid accumulate and lipid fusion in liver parenchyma (*).

Figure C Photomicrograph of the liver shows pancreatic capsule rupture (→) and duct lumen distort (*).

Figure D Photomicrograph of the liver shows granulocytes infiltration (→), twisted duct (⇒), foci necrosis (*), pyknotic nucleus (>>) and cell swelling (>).

Figure E Photomicrograph of the liver shows necrosis of pancreas (*), capsule of pancreas ruptured (>).

Figure F Photomicrograph of the liver shows pancreatic cell shrinkage, lost of the zymogen granule (*), foci necrosis (>) and pyknotic nucleus (→).

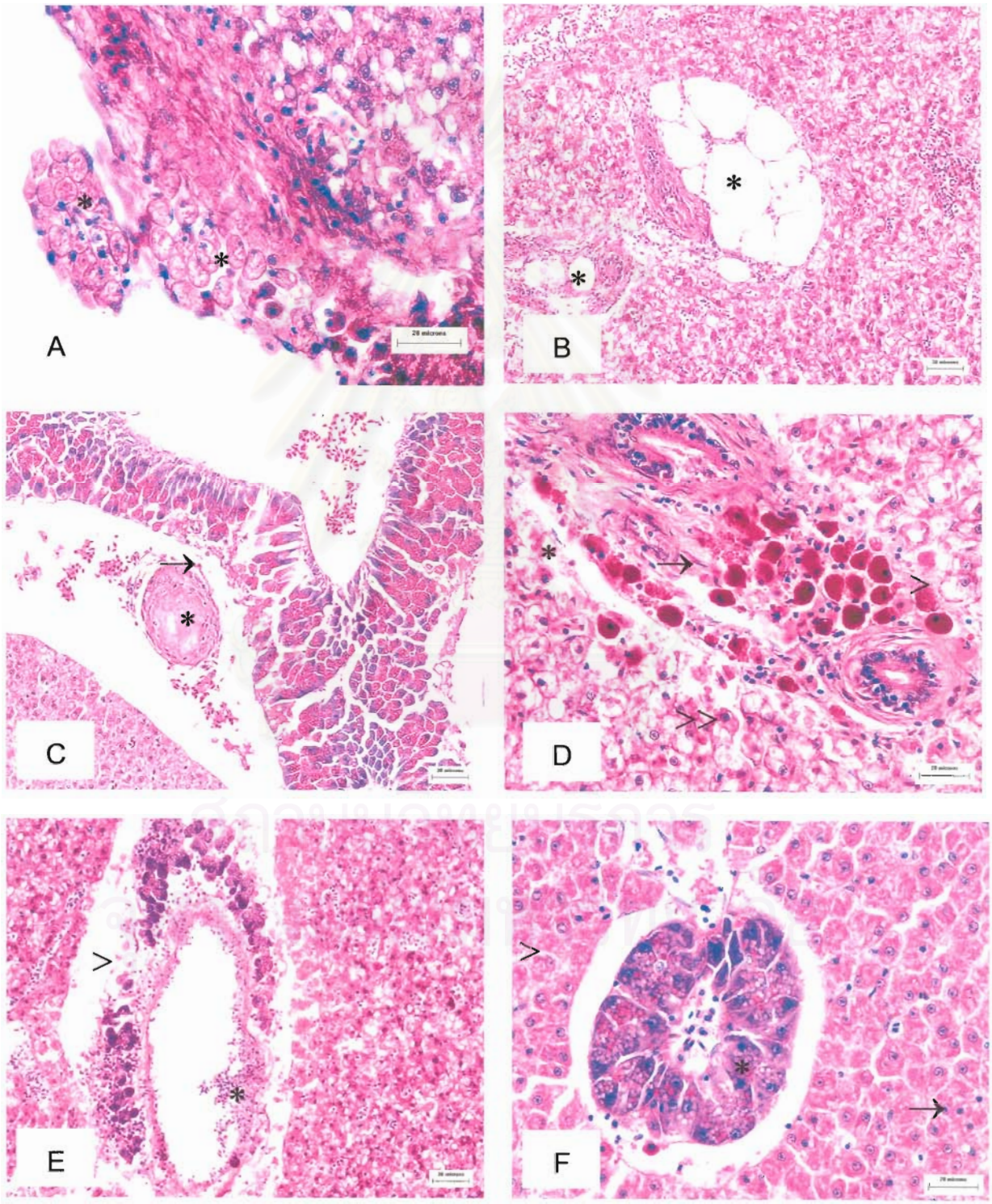

A
B
C
D
E
F

Plate 6

Liver of Klong 7 in rainy season fish

(H&E staining)

Figure A Photomicrograph shows hyalin droplets (→) accumulation in cytoplasm of hepatocytes, eosinophilic cell (>) and pyknotic nucleus (⇒) and foci necrosis (*).

Figure B Photomicrograph shows foci necrosis (*), pyknotic nucleus (→).

Figure C Photomicrograph showing lipid droplets accumulation (→) in cytoplasm and edema hepatocytes (>).

Figure D Photomicrograph showing hyalin droplets (*) deposition in cytoplasm of hepatic cell, pyknotic nucleus (→), karyolysis (⇒) and cell lysis (>).

Figure E Photomicrograph showing lymphocytes (*) infiltration in liver parenchyma.

Figure F Photomicrograph showing granulocytes (→) and lymphocytes (>) infiltration around blood vessels and the thickening of endothelium (*) and foci necrosis (⇒).

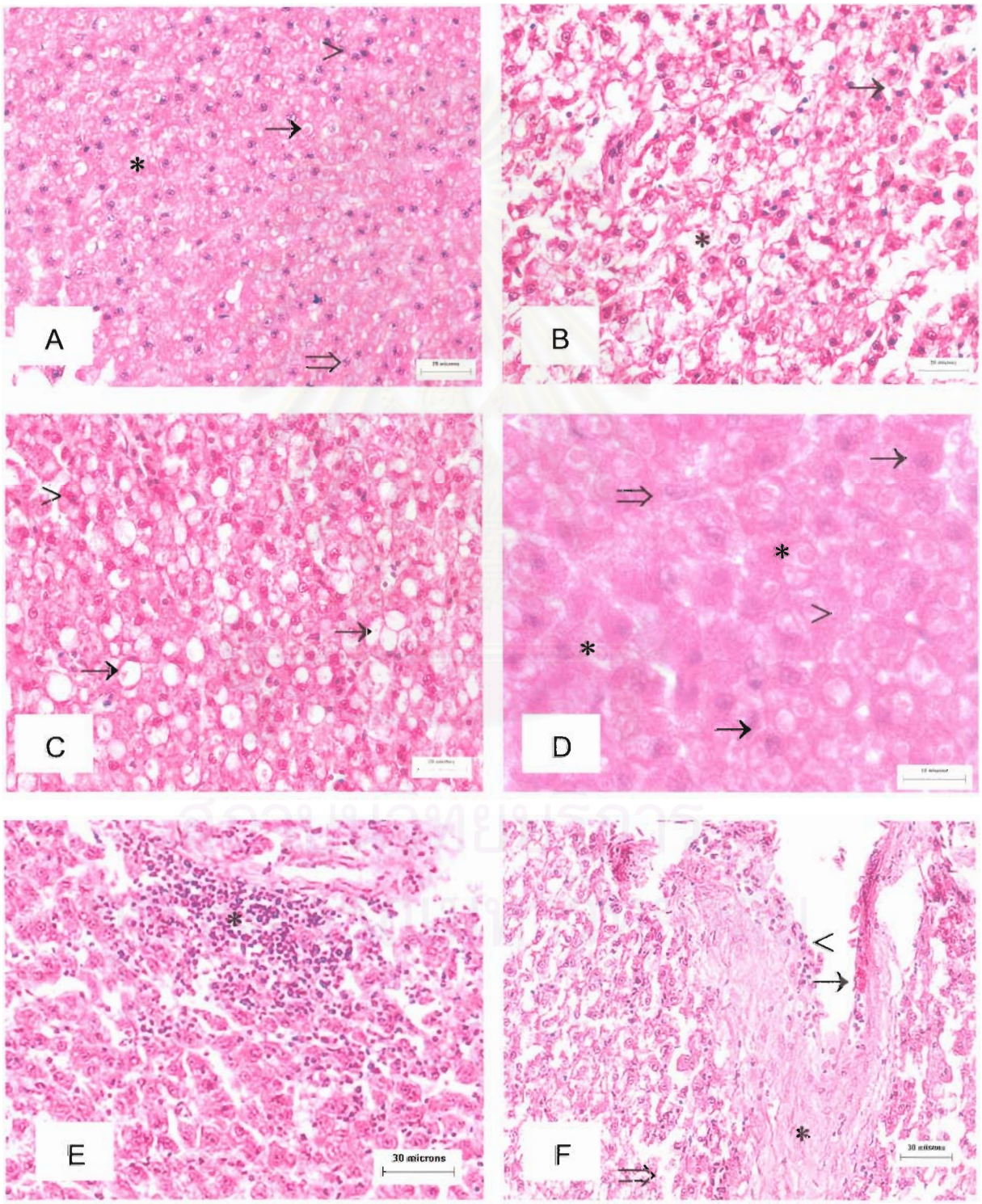

A
B
C
D
E
30 microns
F
30 microns

Plate 7

Liver of Klong 7 in rainy season fish

(H&E staining)

Figure A Photomicrograph shows diffuse necrosis (*), the capsule sloughing (→), eosinophilic area (⇒) and hepatic plate lost of structure.

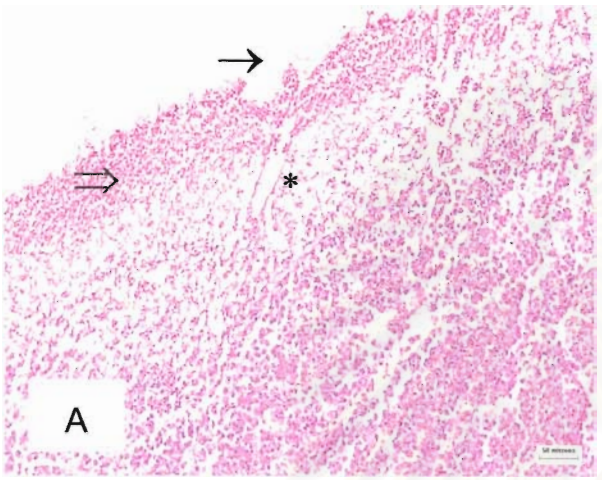
Figure B Photomicrograph shows the sloughing of capsule (>) and widening of sub capsular space (*) of liver tissue and karyolysis (→).

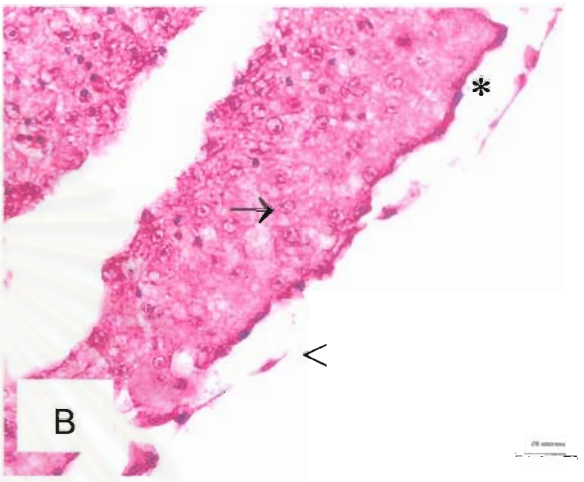
Figure C Photomicrograph shows the sloughing of endothelium of blood vessel (→) and pyknotic nucleus (⇒).

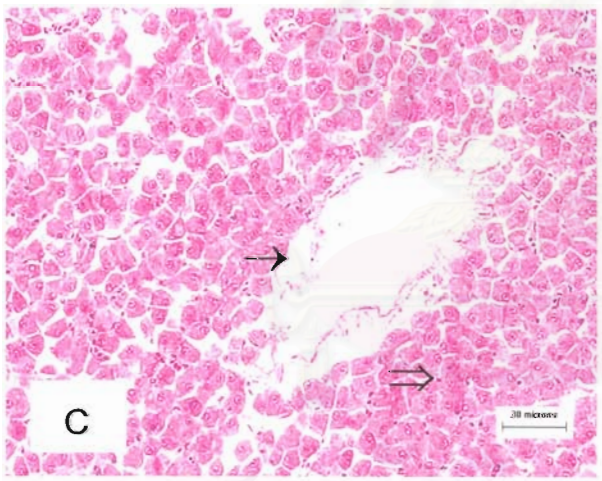
Figure D Photomicrograph shows thickening of endothelium of blood vessel (*) and encapsulated dead cells (>).

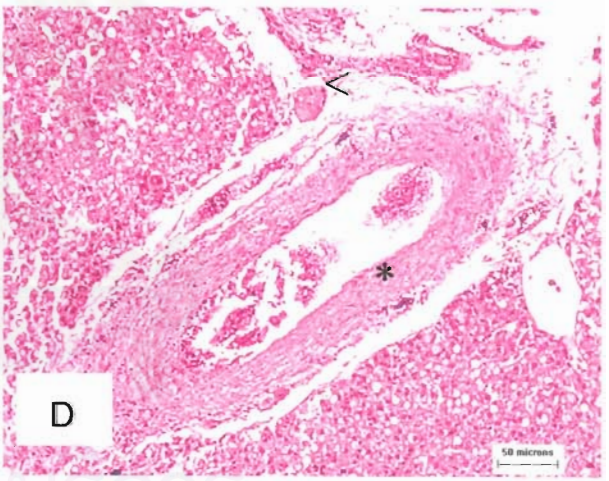
Figure E Photomicrograph shows cell death with capsule (*) and blood congestion.

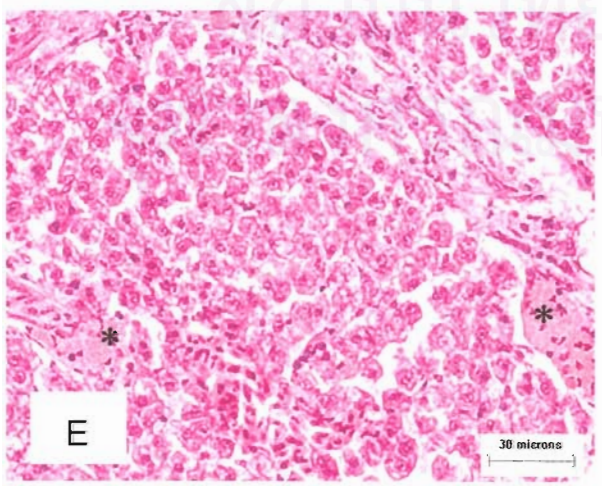
Figure F Photomicrograph shows eosinophilic cells (→) at the area of necrosis.


A


B


C
30 microns


D
50 microns


E
30 microns

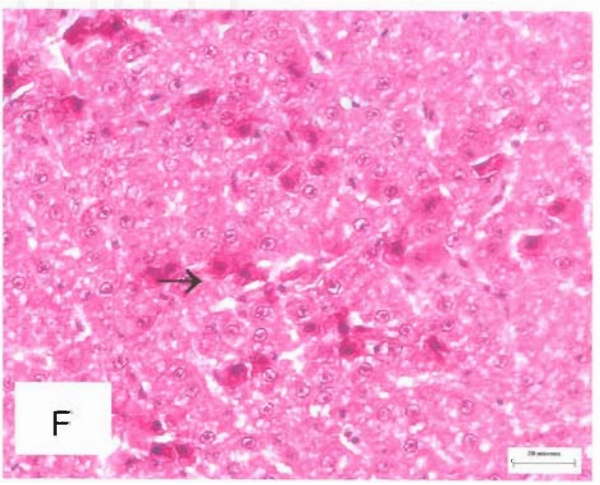

F

Plate 8

Liver of Klong 7 in rainy season fish

(H&E staining)

Figure A Photomicrograph shows the perinuclear chromatin clumping nucleus (→), foci necrosis (*), pyknotic nucleus and karyolytic cell (>).

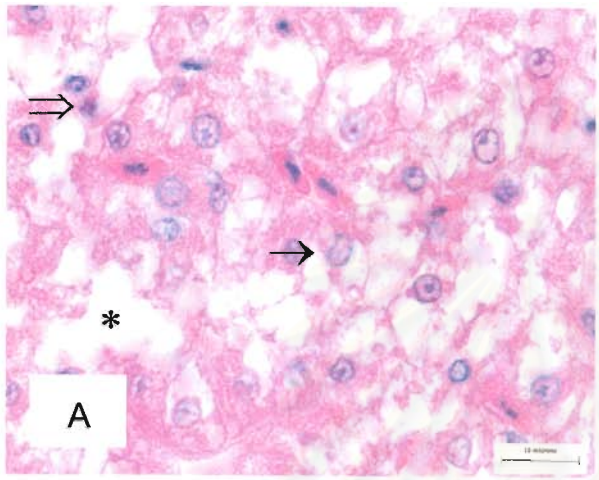
Figure B Photomicrograph shows hyalin droplets accumulation (→), karyolysis (⇒) and binuclear (>).

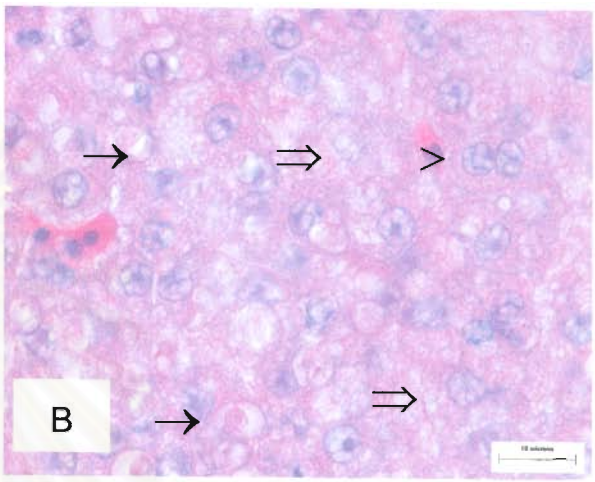
Figure C Photomicrograph shows karyolysis (→), pyknotic nucleus (⇒), cell swelling (>) and cell lysis (*).

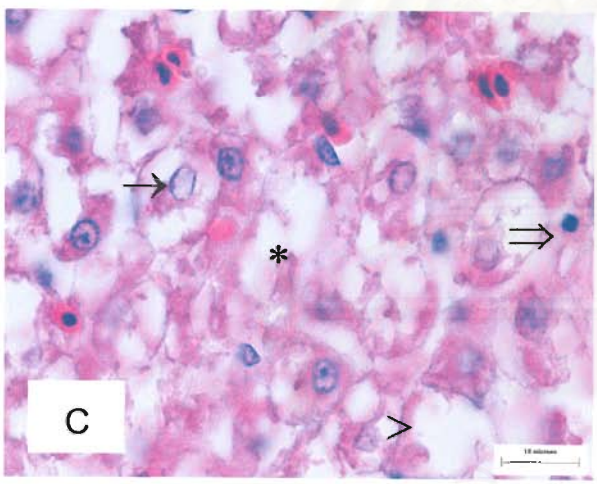
Figure D Photomicrograph shows pyknotic nucleus (→).

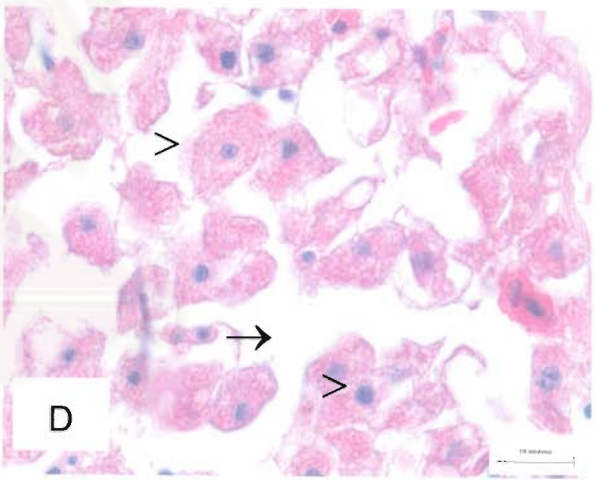
Figure E Photomicrograph shows karyolysis (→), pyknotic nucleus (>>), hyalin droplet deposition (⇒) and foci necrosis (*).

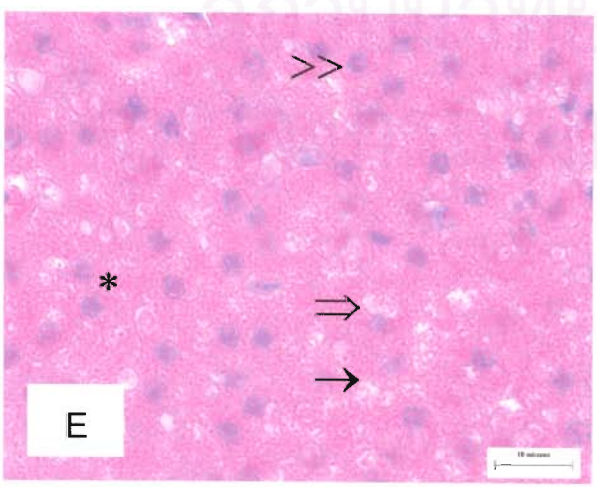
Figure F Photomicrograph shows the ruptured of endothelial lining of central vein (→), foci necrosis (*) around the central vein, edema cell (⇒), pyknotic nucleus (>) and the bile duct (BD) necrosis.


A


B


C


D


E

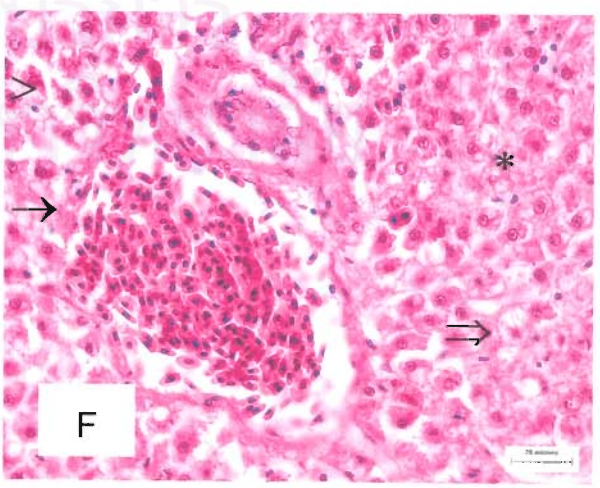

F

Plate 9

Liver of Klong 7 in rainy season fish

(H&E staining)

Figure A Photomicrograph shows blood congestion in central vein, sinusoid and in hepatic parenchyma (*).

Figure B Photomicrograph shows group of necrotic cells and cellular debris in blood vessel (→).

Figure C Photomicrograph showing pancreatic cell shrinkage (⇒), blood congestion in blood vessels of pancreas (*) and disarrangement of pancreatic architecture (→).

Figure D Photomicrograph showing encapsulated of necrotic cells (*) in pancreas and pancreatic cells necrosis (→).

Figure E Photomicrograph of the liver and pancreas shows the distortion of pancreatic tissue in liver parenchyma (*).

Figure F Photomicrograph showing necrosis of pancreatic cells (*) and lost of zymogen granule in pancreas (→).

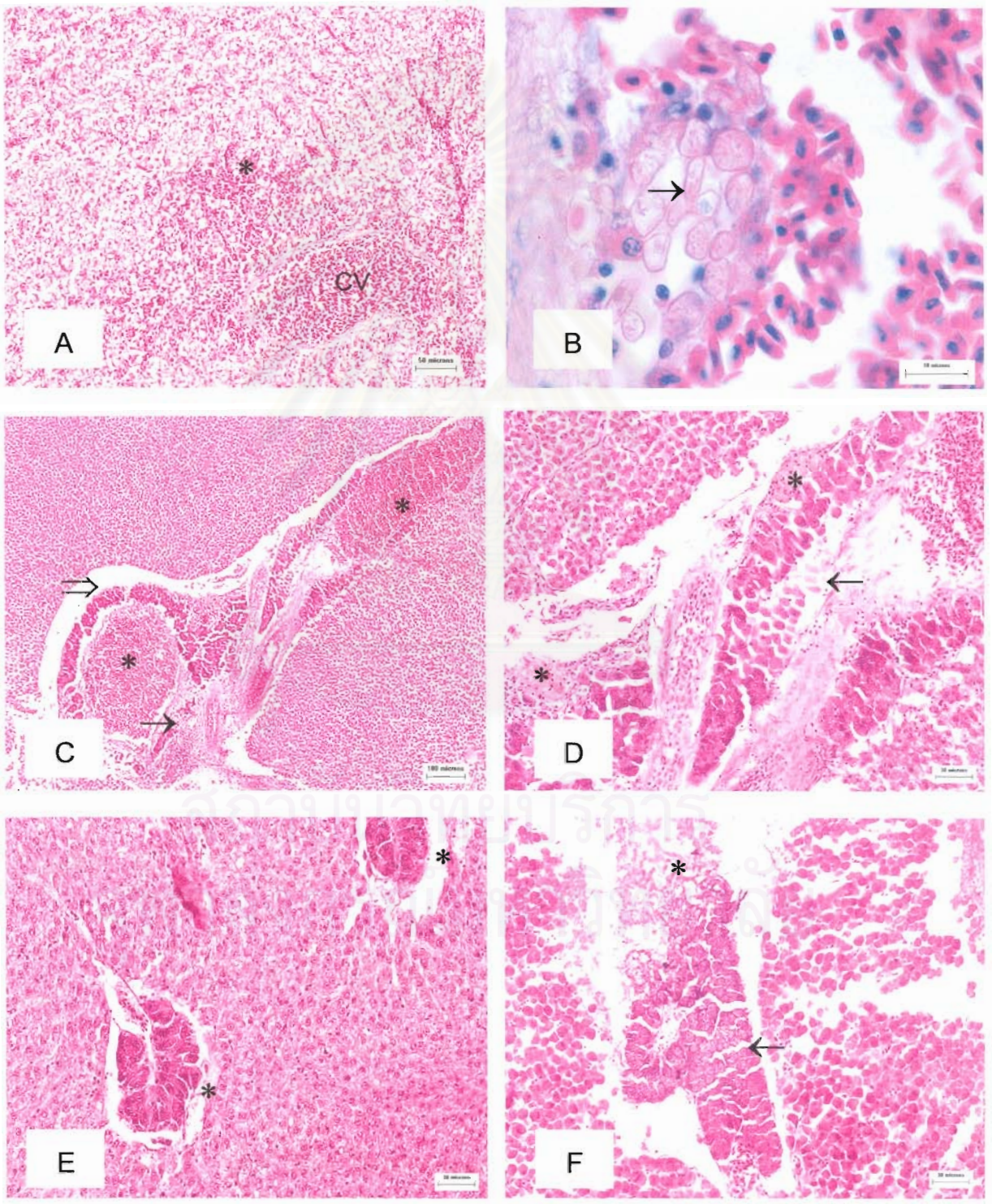

A
CV
B
C
D
E
F

Plate 10

Comparison of liver tissue between dry season fish and rainy season fish (H&E staining)

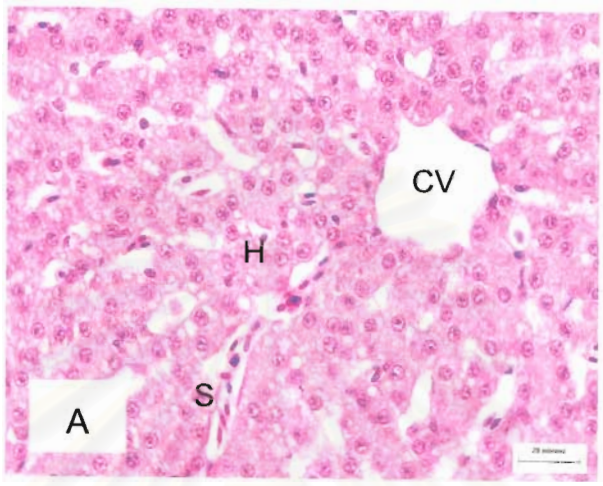
Figure A Photomicrograph shows the normal liver that comprise of hepatic plate, the polygonal hepatocytes are arranged between sinusoid (S) that connected with central vein (CV). Lipid droplets were accumulation in cytoplasm of hepatocytes (*).

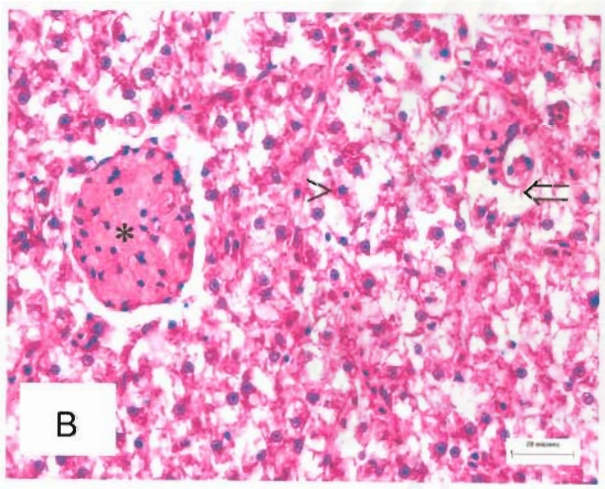
Figure B Photomicrograph of dry season fish liver shows encapsulated of aggregated necrotic cells (*) in liver parenchyma, cell necrosis (⟹) and pyknotic cell (>).

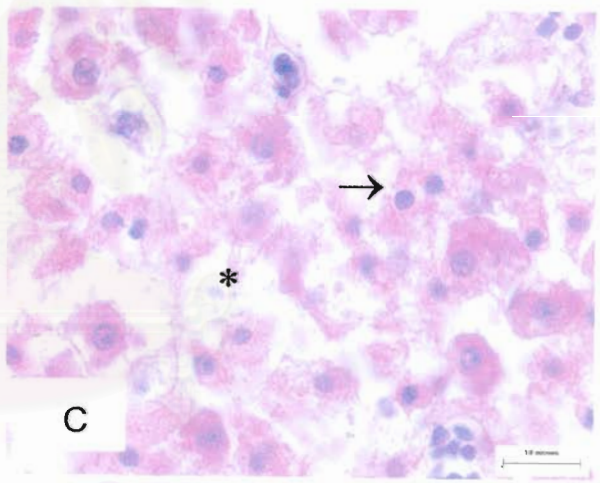
Figure C Photomicrograph of dry season fish liver showing pyknotic nucleus (→) and cell necrosis (*).

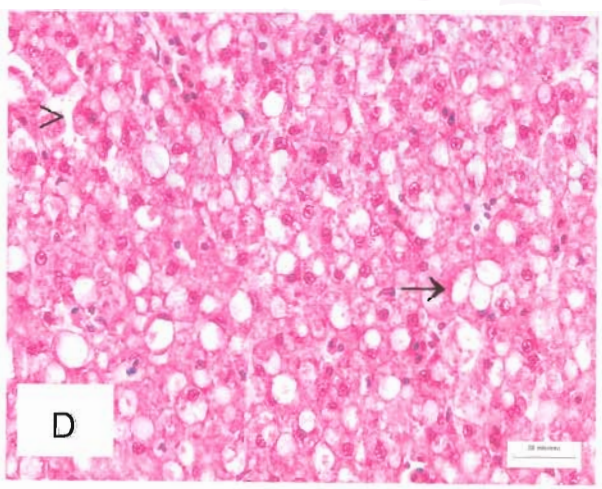
Figure D Photomicrograph of rainy season fish liver showing lipid droplets accumulation (→) in cytoplasm and edema cell (>).

Figure E Photomicrograph of rainy season fish liver shows the perinuclear chromatin clumping nucleus of hepatocytes (→), foci necrosis (*), pyknotic nucleus and karyolysis (>).


CV
H
S
A


B


C


D

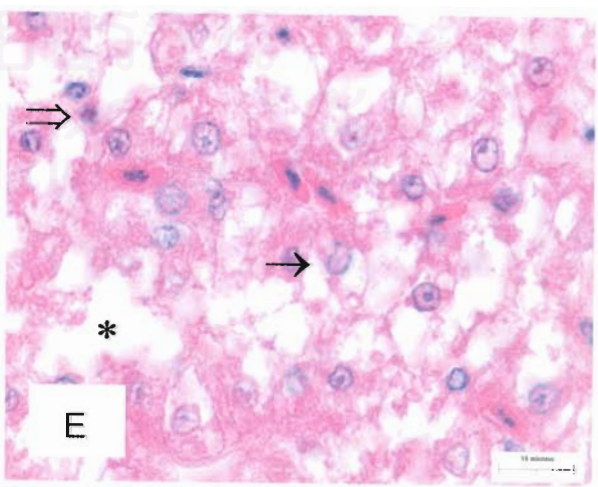

E

Plate 11

Comparison the liver of Klong 7 in rainy season fish and dry season fish (Oil red O and PAS staining)

Figure A Photomicrograph of the Oil red O Staining of control liver showing the small lipid droplets deposit in hepatocytes of liver parenchyma.

Figure B Photomicrograph of the PAS staining of control liver showing the positive PAS stains.

Figure C Photomicrograph of the Oil red O staining of Klong 7 liver in dry season showing large size of lipid droplets deposit in the liver tissue and less number density than control liver.

Figure D Photomicrograph of the PAS staining of Klong 7 liver in dry season shows positive PAS stains.

Figure E Photomicrograph of the Oil red O staining of Klong 7 liver in rainy season showing moderately size of lipid droplets and more density than in dry season deposit in the liver tissue.

Figure F Photomicrograph of the PAS staining of Klong 7 liver in rainy season showing positive PAS stains.

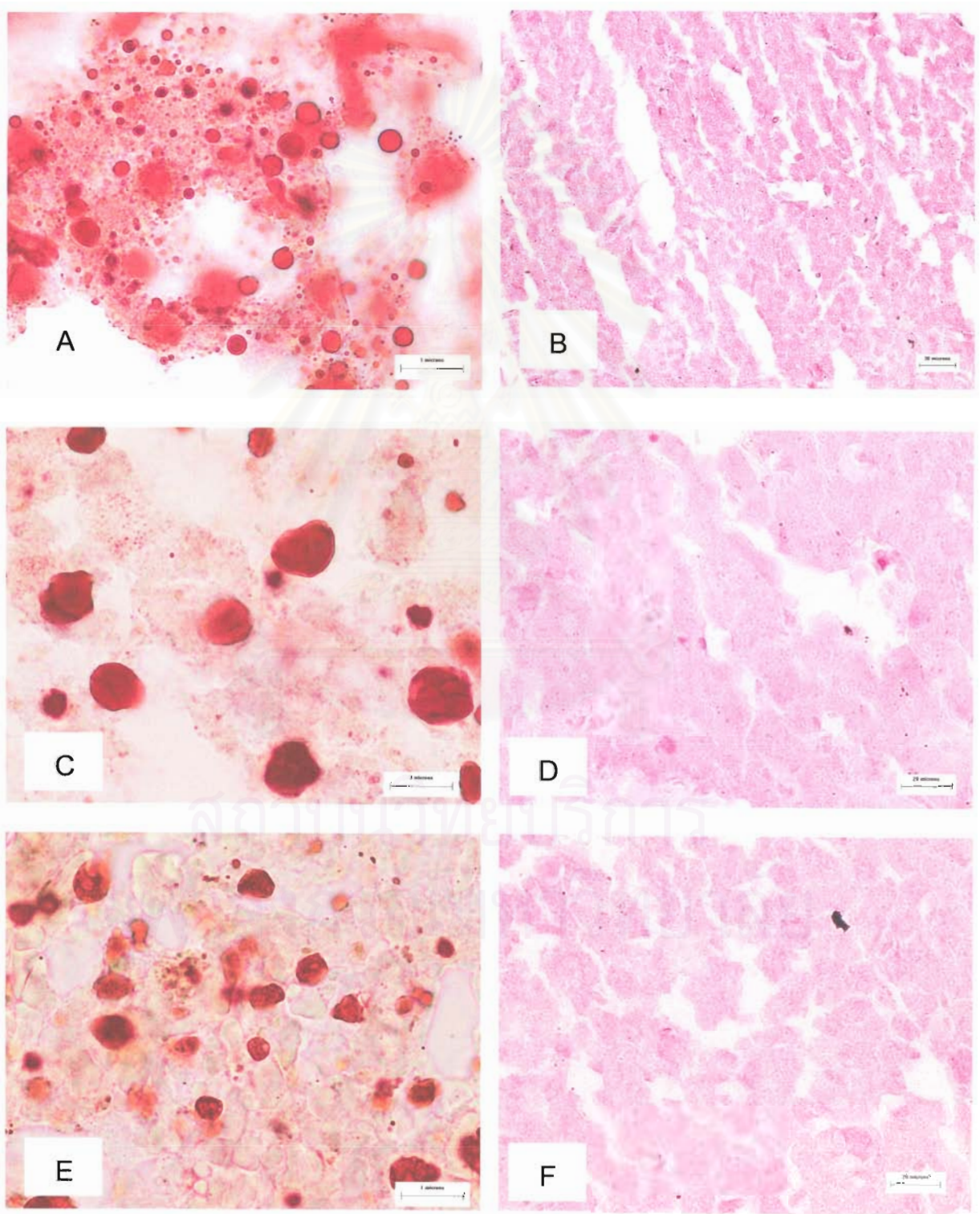

A
B
C
D
E
F

Plate 12

Liver of the 0.06 ppb endosulfan exposed liver

(H&E staining)

Figure A Photomicrograph of the control liver shows normal structure.

Figure B Photomicrograph of the endosulfan treated liver shows blood congestion (*) and shrinkage of pancreas (→).

Figure C Photomicrograph of the endosulfan treated liver shows the endothelial lining ruptured (→), lipid vacuoles accumulation (*) and foci necrosis (⇒).

Figure D Photomicrograph of the endosulfan treated liver shows epithelium lining of pancreatic capsule (→) and blood vessel of pancreas ruptured (>), necrosis of pancreatic cells (*), lost ofthe zymogen in pancreas abnormal (⇒) and duct (D) distorted.

Figure E Photomicrograph of the endosulfan treated liver shows macrophage infiltration in pancreas (→), lost of zymogen in pancreas l (*), sloughing of endothelium of blood vessel in pancreas (>) and pancreatic cell necrosis (⇒).

Figure F Photomicrograph of the endosulfan treated liver shows capsule ruptured (→).

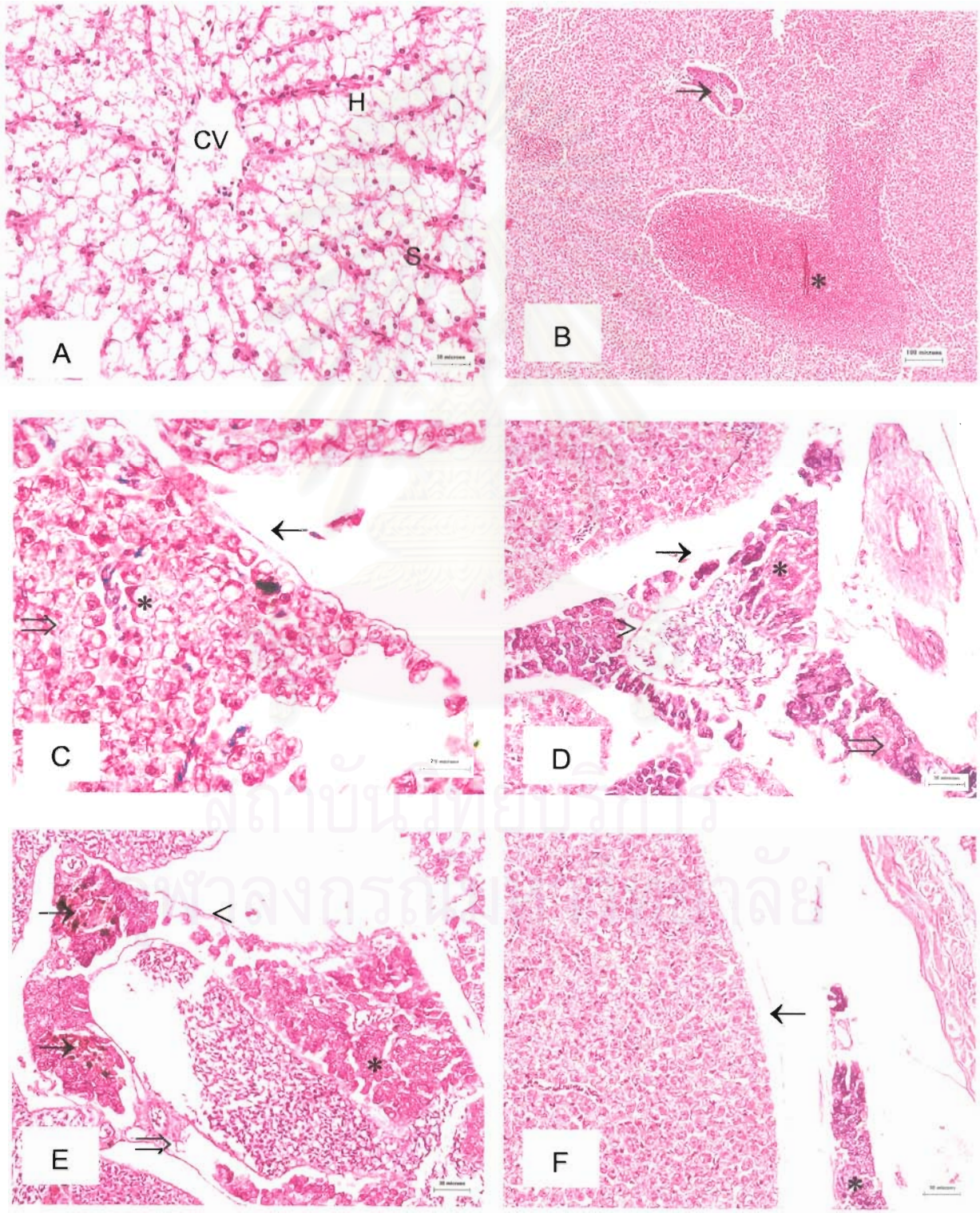

A
CV
H
S
B
C
D
E
F

Plate 13

Liver of the 0.25 ppb endosulfan exposed liver

(H&E staining)

Figure A Photomicrograph of control liver shows normal structure.

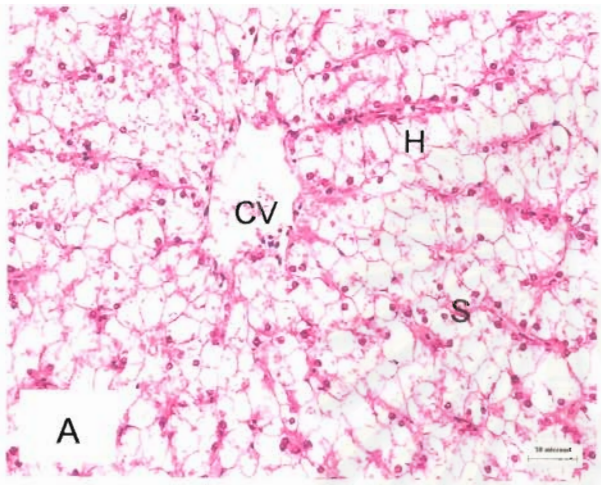
Figure B Photomicrograph of the endosulfan treated liver shows the sloughing of endothelium in central vein (→) and cellular debris (*) in central vein, hyalin droplets accumulation (⇒), cell necrosis (>) and macrophage infiltration (>>).

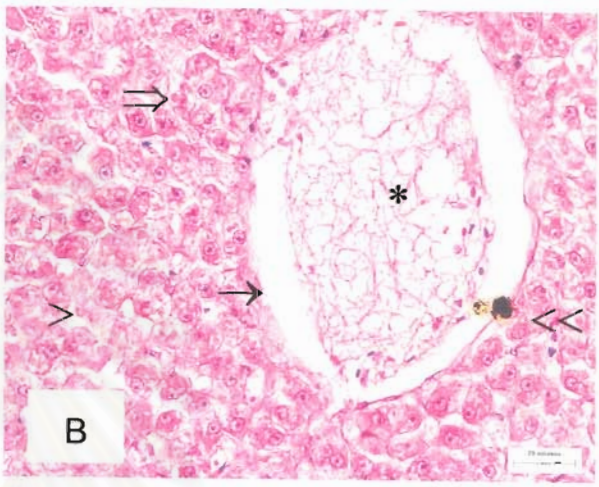
Figure C Photomicrograph of the endosulfan treated liver shows hyalin droplets (→) deposition in cytoplasm, pyknotic nucleus (>>), cell necrosis (*) around the central vein and karyolysis (>).

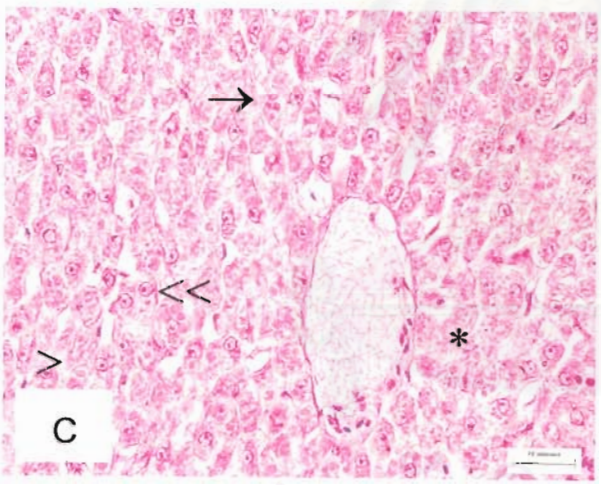
Figure D Photomicrograph of the endosulfan treated liver shows pancreas (P) and duct (D) lumen twisted and pancreatic cells necrosis.

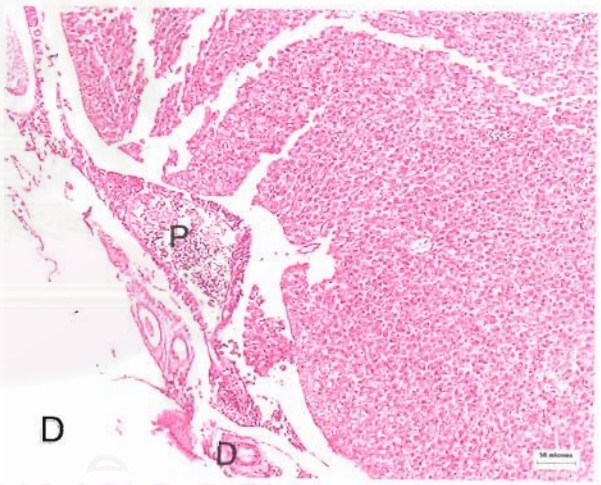
Figure E Photomicrograph of the endosulfan treated liver shows red blood cell swollen (→), cell necrosis (*) and eosinophilic cell (>).

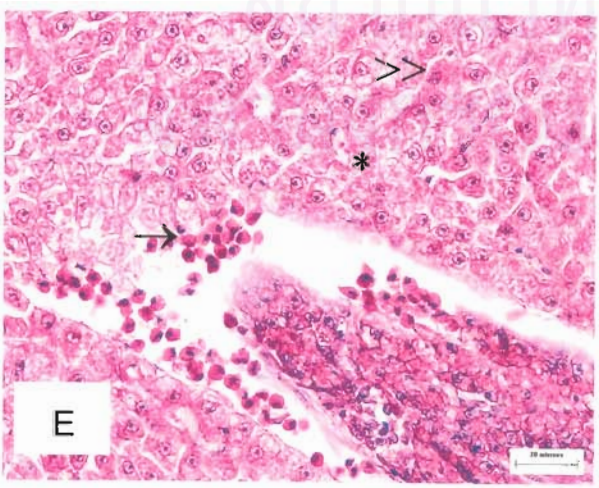
Figure F Photomicrograph of the endosulfan treated liver shows macrophage infiltration (→) in pancreas, pancreas lost of architecture and coat of red blood cell in blood vessel of pancreas (*).


H
CV
S
A


*
B


*
C


P
D
D


*
E

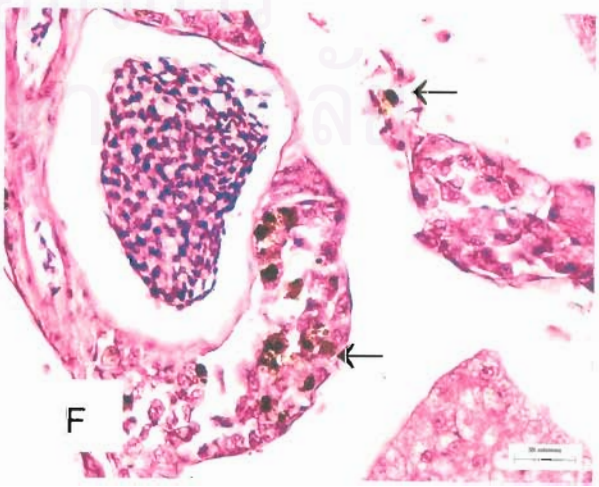

F

Plate 14

Liver of the 0.50 ppb endosulfan exposed liver

(H&E staining)

Figure A Photomicrograph of the control liver shows normal structure.

Figure B Photomicrograph of the endosulfan treated liver shows blood congestion in central vein and red blood cell not cytoplasm (*), red blood cell lysis endothelium of blood vessel ruptured (→), diffuse necrosis (⇒) and foci necrosis (>).

Figure C Photomicrograph of endosulfan treated liver shows the separation of endothelial layer of blood vessel (→), lipid vacuole accumulation (>) and foci necrosis (*) around blood vessel.

Figure D Photomicrograph of endosulfan treated liver shows red blood cell swelling (→), foci necrosis (*), pancreas necrosis (⇒), edema hepatocytes (>) and blood congestion in pancreas (>>).

Figure E Photomicrograph of endosulfan treated liver shows foci necrosis (*), blood congestion in central vein (⇒), sinusoid dilation (S) and hyalin droplets deposition in hepatic cells (→).

Figure F Photomicrograph of endosulfan treated liver shows diffuse necrosis (*) of hepatocytes and pancreatic cells necrosis (→).

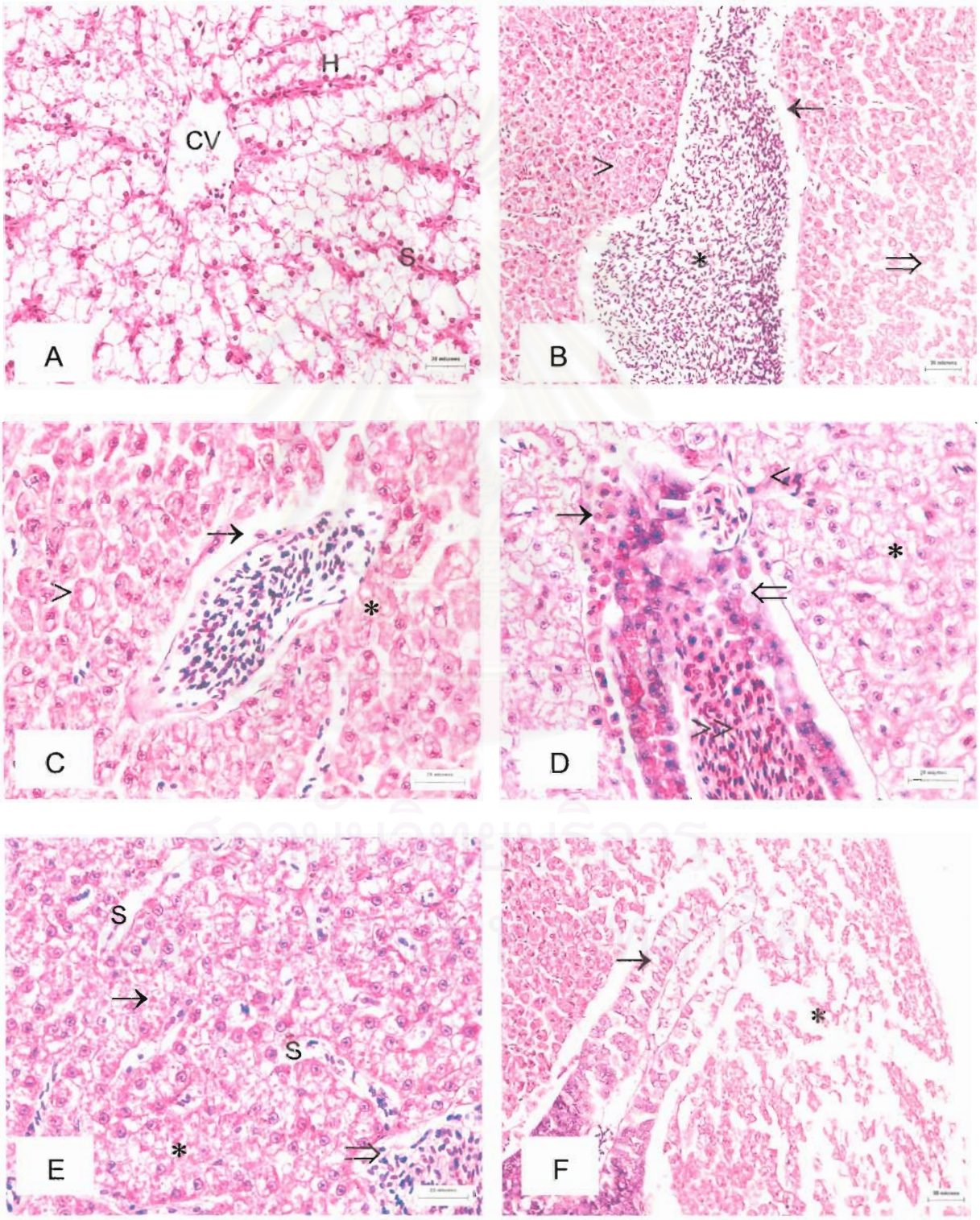

H
CV
S
A
B
C
D
S
S
E
F

Plate 15

Liver of the 1 ppb endosulfan exposed liver

(H&E staining)

Figure A Photomicrograph of the control liver shows normal structure.

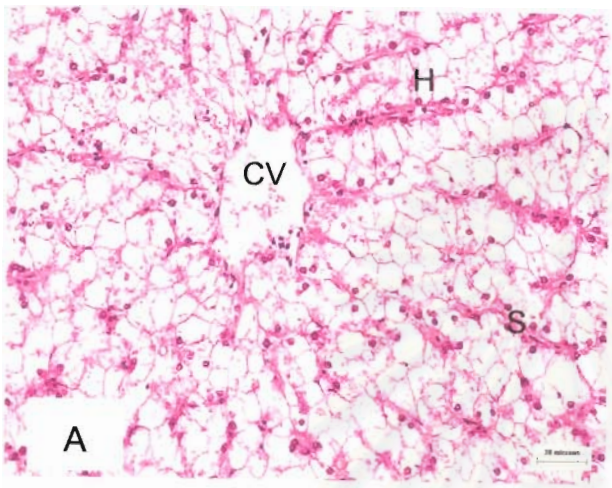
Figure B Photomicrograph of the endosulfan treated liver shows endothelium lining of blood vessel ruptured and separated from liver parenchyma (→), necrotic cells in blood vessel (*), encapsulated cell debris in blood vessel(⇒) and foci necrosis (>>).

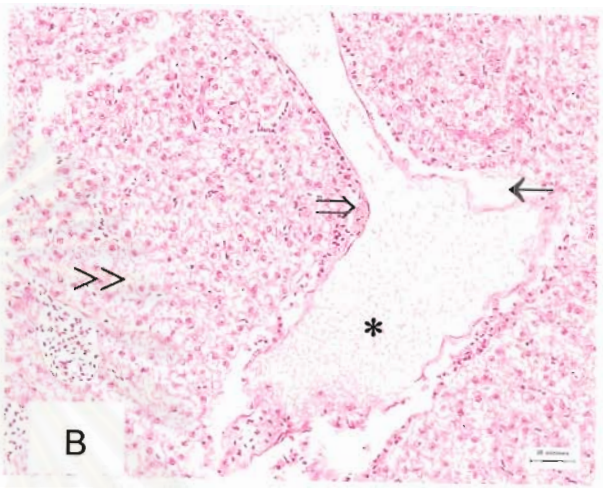
Figure C Photomicrograph of the endosulfan treated liver shows diffuse necrosis (*) in liver parenchyma and macrophage infiltration (→).

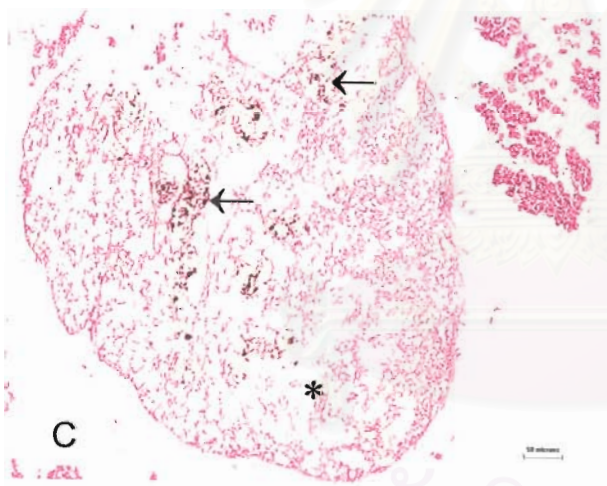
Figure D Photomicrograph of the endosulfan treated liver shows capsule of liver sloughing (→), pyknotic nucleus (>) cell necrosis (*) and bile duct (BD) distorted.

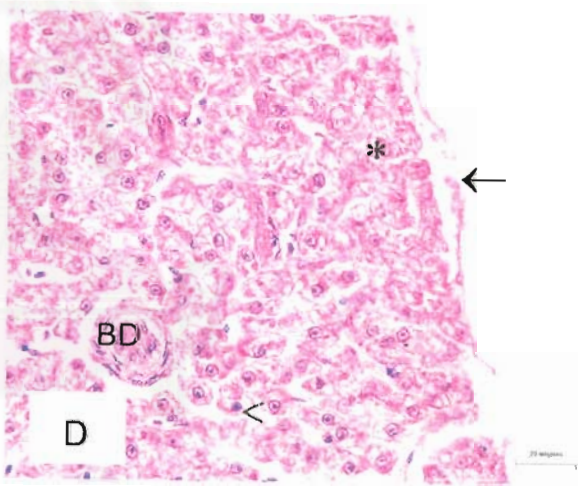
Figure E Photomicrograph of the endosulfan treated liver shows blood congestion (*) in central vein, hyalin deposit (→), cell necrosis (⇒) around the central vein and bile duct (BD).

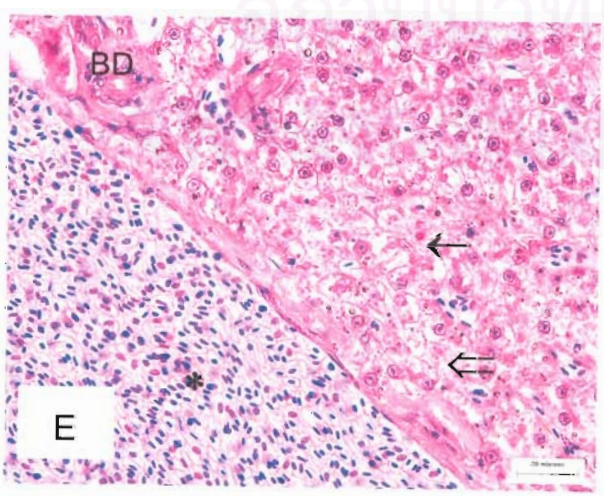
Figure F Photomicrograph of the endosulfan treated liver shows bile duct shrinkage (BD), blood congestion (*) and foci necrosis (⇒).


H
CV
S
A


B


C


BD
D


BD
E

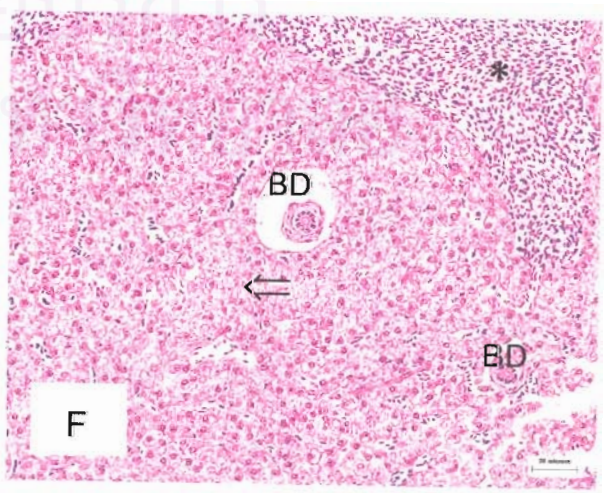

BD
BD
F

Plate 16

Liver of endosulfan exposed liver

(Oil red O staining)

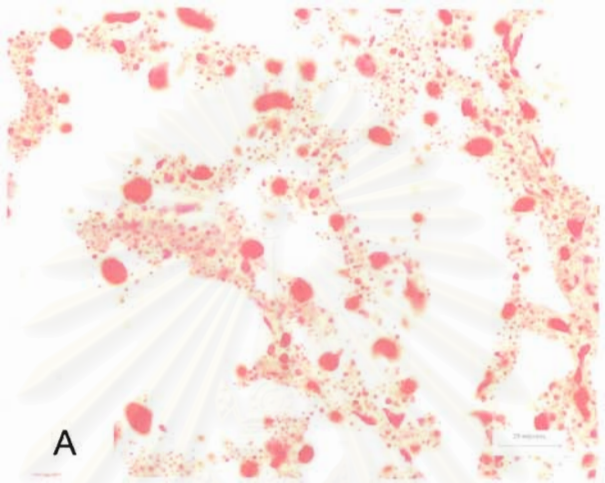
Figure A Photomicrograph of control liver shows small lipid droplet distribute through out liver parenchyma.

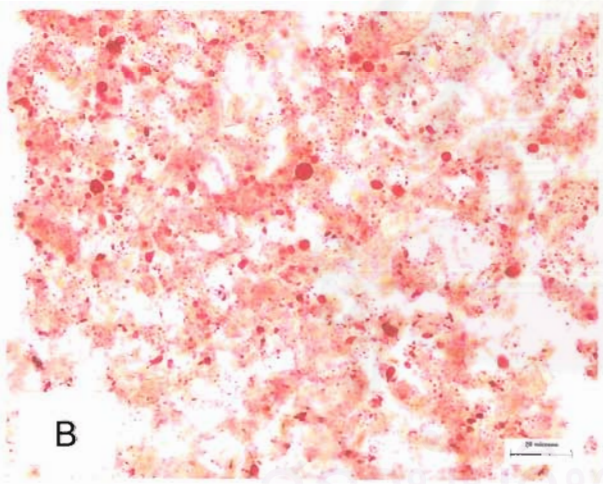
Figure B Photomicrograph of 0.06 ppb endosulfan treated liver shows smaller lipid droplet and less density lipid droplets than the control liver.

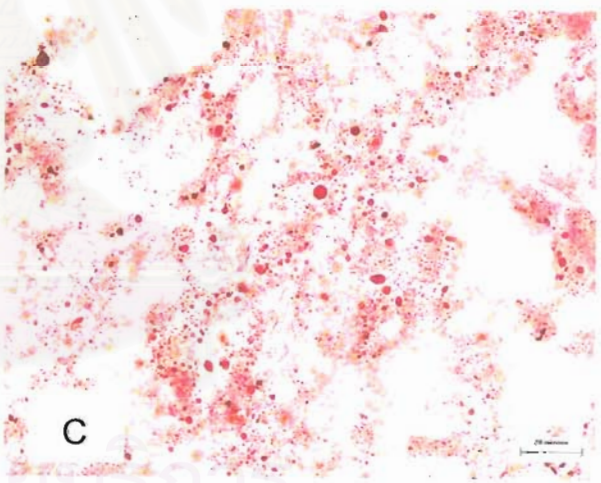
Figure C Photomicrograph of 0.25 ppb endosulfan treated shows smaller lipid droplet and less density of lipid droplet than the control liver.

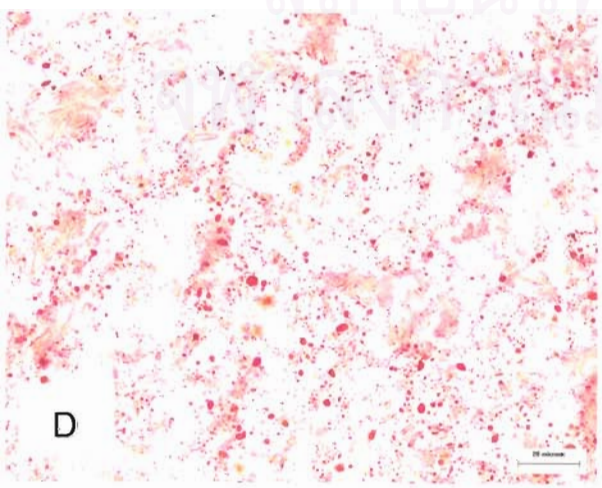
Figure D Photomicrograph of 0.50 ppb endosulfan treated shows smaller lipid droplet and less density of lipid droplet than the control liver.

Figure E Photomicrograph of 1 ppb endosulfan treated shows smaller lipid droplet and less density of lipid droplet than the control liver.


A


B


C


D

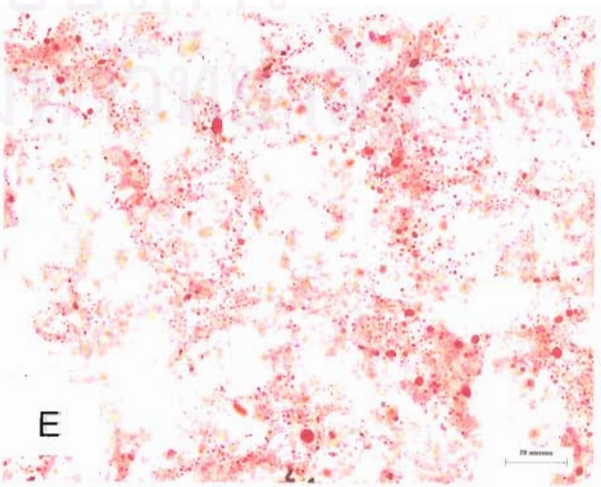

E

Plate 17

Liver of endosulfan exposed liver

(PAS staining)

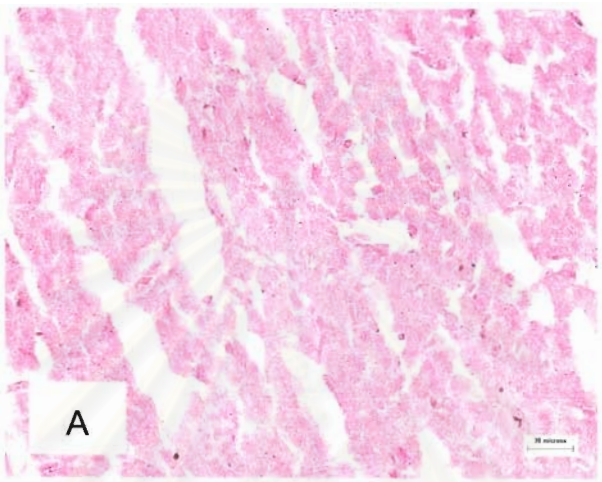
Figure A Photomicrograph of the positive Pas staining of control liver shows the deposition of glycogen in liver tissue.

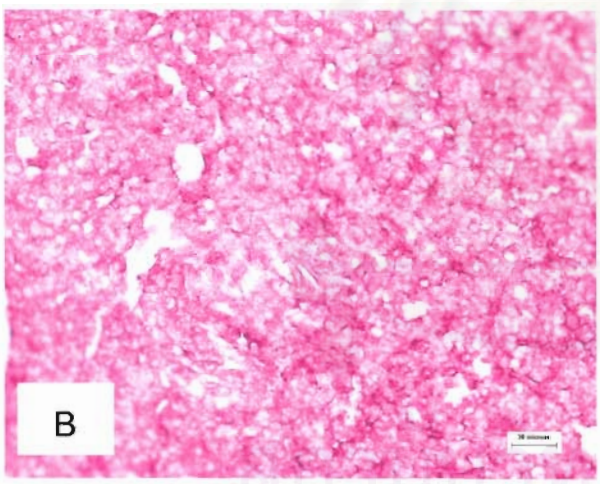
Figure B Photomicrograph of the 0.06 ppb endosulfan treated liver shows the more positive staining of glycogen deposit than the control liver.

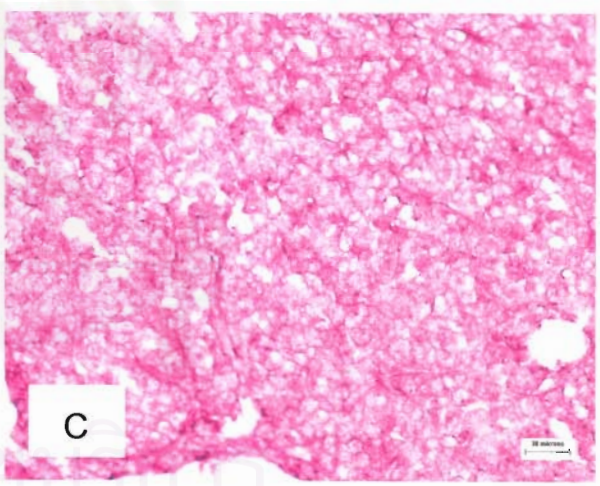
Figure C Photomicrograph of the 0.25 ppb endosulfan treated liver shows the more positive staining of glycogen deposition than the control liver.

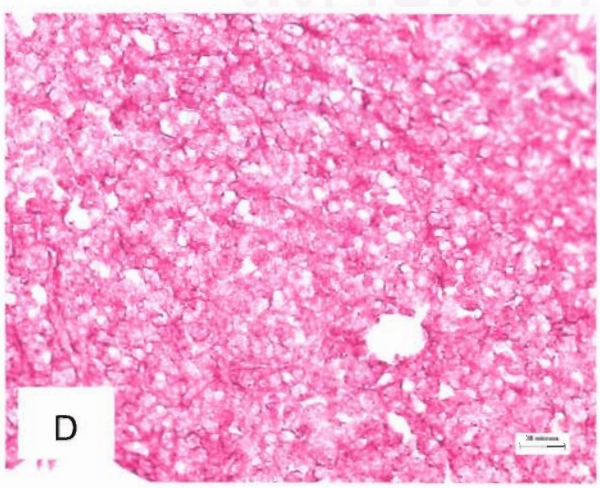
Figure D Photomicrograph of the 0.50 ppb endosulfan treated liver shows the more positive staining of glycogen deposition than the control liver.

Figure E Photomicrograph of the 1 ppb endosulfan treated liver shows the most positive staining of glycogen deposition in liver parenchyma.


A


B


C


D

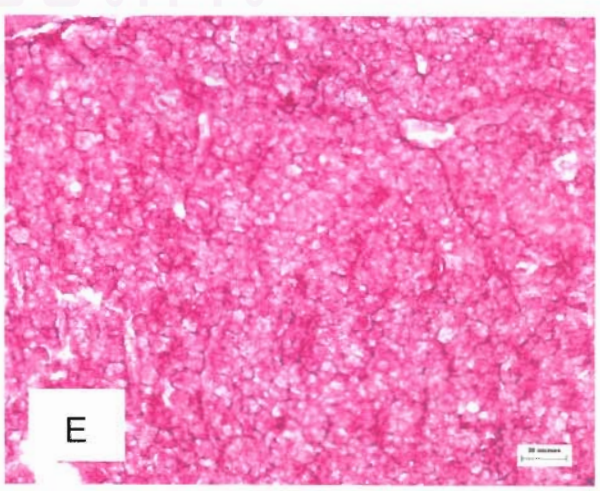

E

Plate 18

Comparison of livers between dry season fish, rainy season fish and endosulfan treated fish (H&E staining)

Figure A Photomicrograph of the control liver shows normal hepatic plate, the polygonal hepatocytes are arranged between sinusoid (S) that connected with central vein (CV) and have lipid droplets accumulation in cytoplasm of hepatocytes (*).

Figure B Photomicrograph of the liver of Klong 7 in dry season fish shows encapsulate aggregated of dead cells (*) in liver parenchyma, cell necrosis (⟹) and pyknotic cell (>).

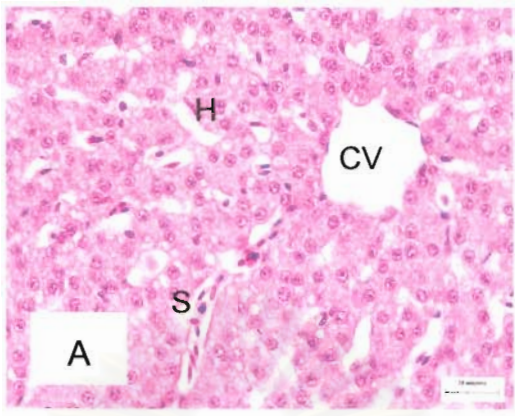
Figure C Photomicrograph the liver of Klong 7 in rainy season fish shows pyknotic nucleus (→), cell necrosis (*).

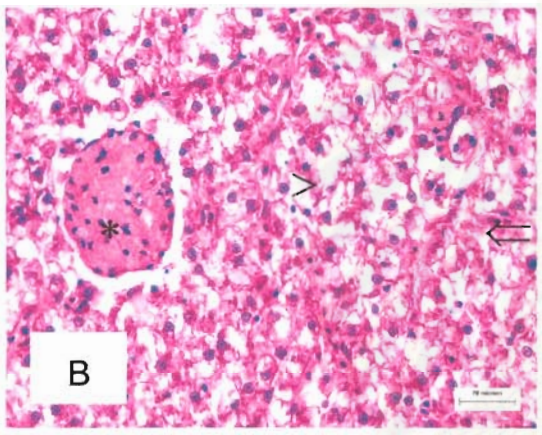
Figure D Photomicrograph of the 0.06 ppb endosulfan treated liver shows the detachment of capsule (→) and cell necrosis (*).

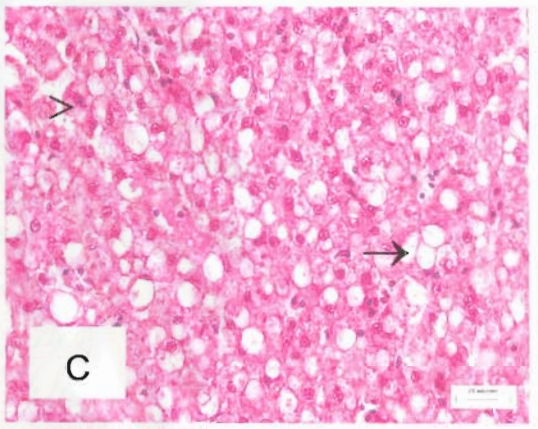
Figure E Photomicrograph of the 0.25 ppb endosulfan treated liver shows hyalin droplets deposition (→), foci necrosis (*) and karyolytic cell (<<).

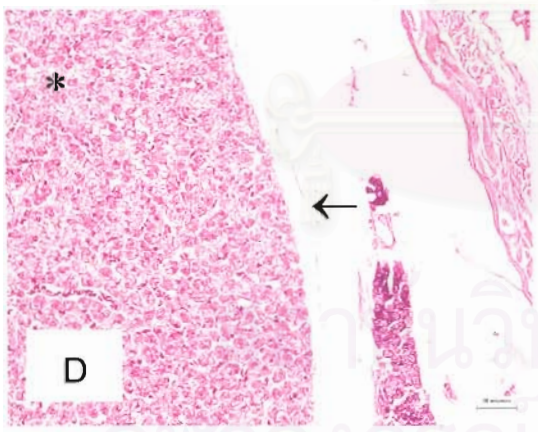
Figure F Photomicrograph of the 0.50 ppb endosulfan treated liver shows the detachment of endothelium of blood vessel (→), lipid vacuole accumulation (>) and foci necrosis (*) around blood vessel.

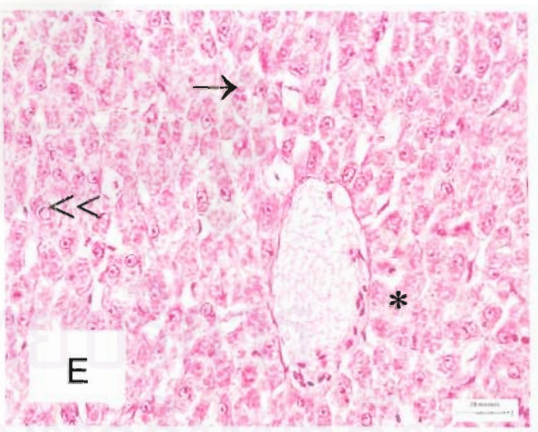
Figure G Photomicrograph of the 1 ppb endosulfan treated liver shows diffuse necrosis (*) through the liver tissue and macrophage infiltration (→).

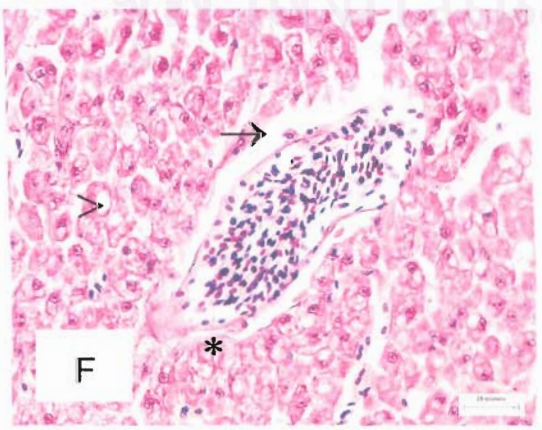

H
CV
S
A


B


C


D


E


F

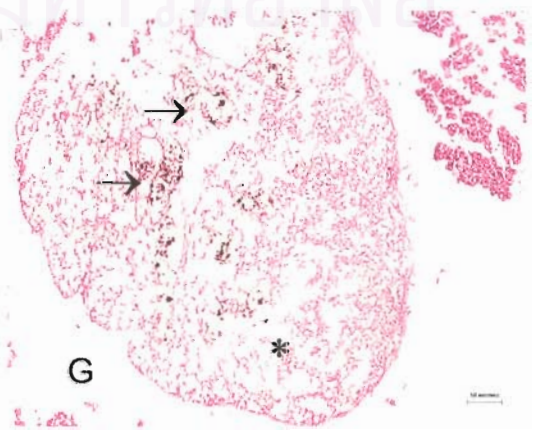

G

Plate 19

Comparison of livers between Klong 7 in dry season fish, rainy season fish and endosulfan treated fish (Oil red O staining)

Figure A Photomicrograph of the Oil red O staining liver of control fish showing small size of lipid deposition through out the liver tissue.

Figure B Photomicrograph of the Klong 7 in dry season liver showing the large size of lipid droplets deposition in the liver tissue,

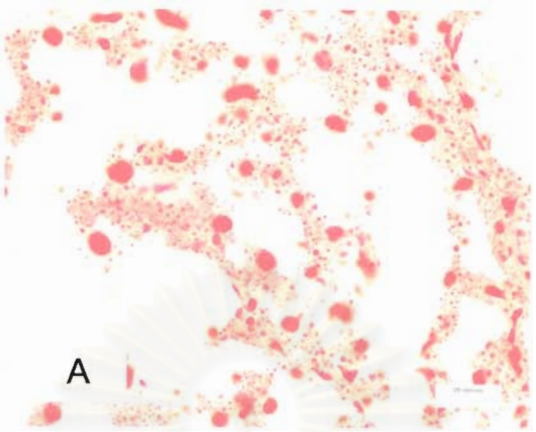
Figure C Photomicrograph of the Klong 7 in rainy season liver showing the medium size of lipid droplets deposition in the liver tissue and less density of droplets than the control liver.

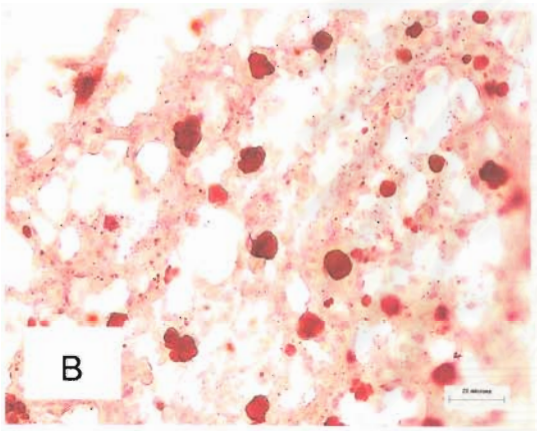
Figure D Photomicrograph of the 0.06 ppb endosulfan treated liver showing the smaller size of lipid droplets and less density of droplets than the control liver.

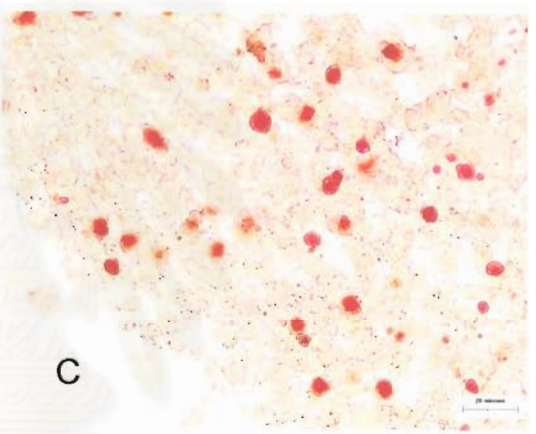
Figure E Photomicrograph of the 0.25 ppb endosulfan treated liver showing the smaller size of lipid droplets and less density of droplets than the control liver.

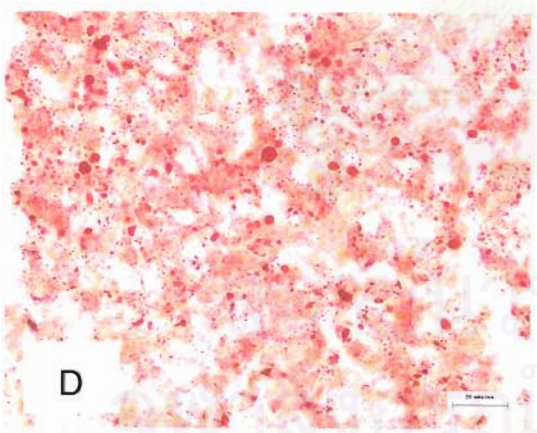
Figure F Photomicrograph of the 0.50 ppb endosulfan treated liver showing the very small size of lipid droplets and the least density of lipid droplets.

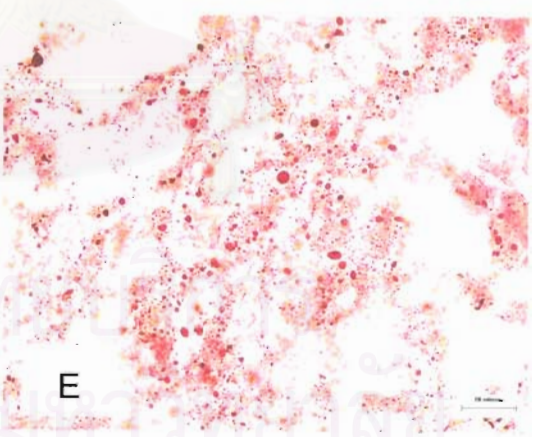
Figure G Photomicrograph of the 1 ppb endosulfan treated liver showing the very small size of lipid droplets and the least density of lipid droplets.

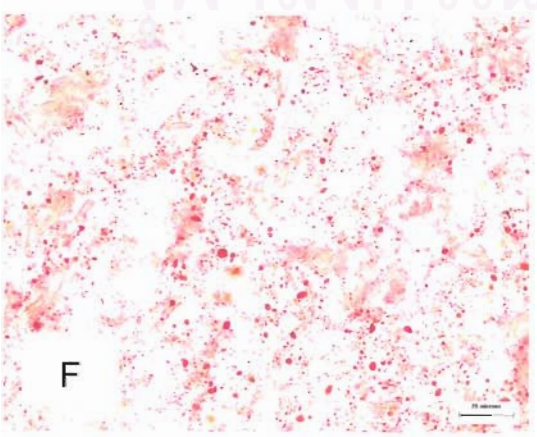

A


B


C


D


E


F

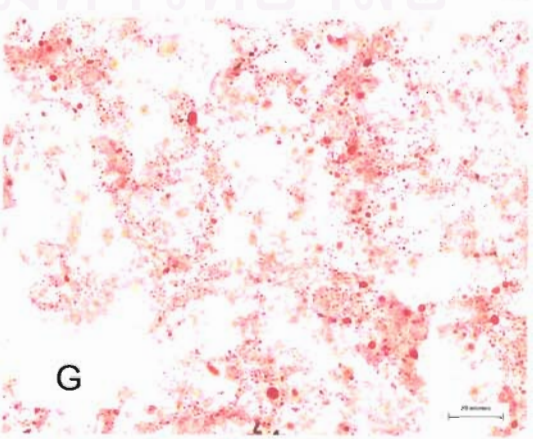

G

Plate 20

Comparison between the liver tissue of Klong 7 dry season fish, rainy season fish and endosulfan treated fish (PAS staining)

Figure A Photomicrograph of control liver shows the positive PAS staining of glycogen deposition in liver parenchyma.

Figure B Photomicrograph of Klong 7 in dry season liver shows positive PAS staining with the deposition of glycogen in liver tissue.

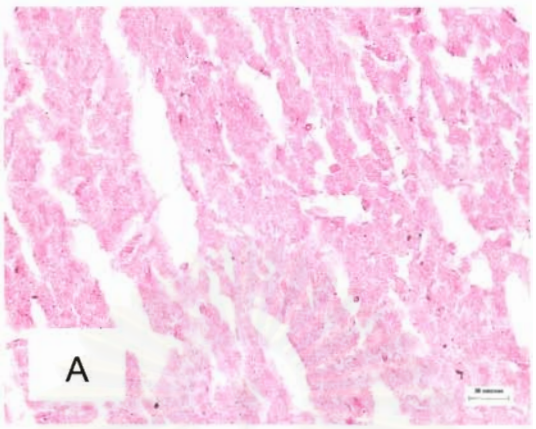
Figure C Photomicrograph of Klong 7 rainy season liver shows less positive PAS staining of glycogen deposition in liver tissue when compare to control liver.

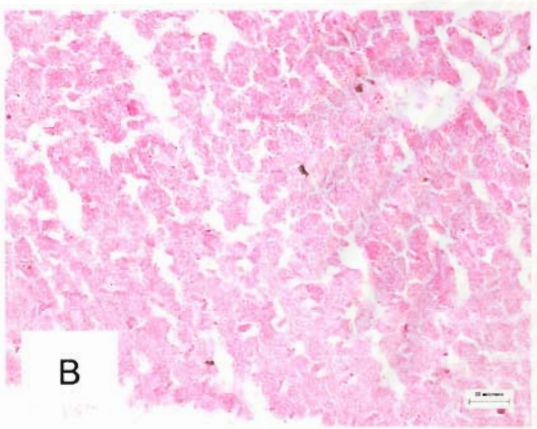
Figure D Photomicrograph of the 0.06 ppb endosulfan treated liver shows more positive PAS staining of glycogen deposition in liver tissue when compare to control liver.

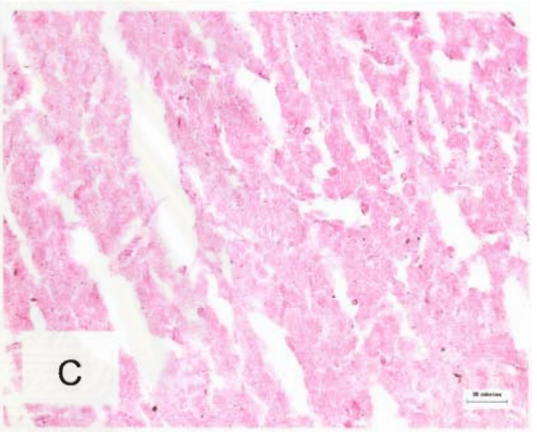
Figure E Photomicrograph of the 0.25 ppb endosulfan treated liver shows more positive PAS staining of glycogen deposition in liver tissue when compare to control liver.

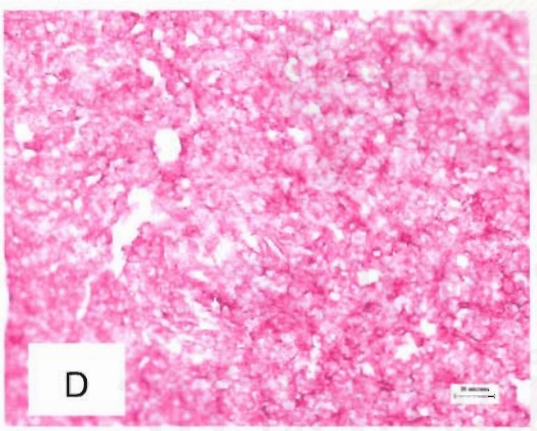
Figure F Photomicrograph of the 0.50 ppb endosulfan treated liver shows more positive PAS staining of glycogen deposition in liver tissue when compare to control liver.

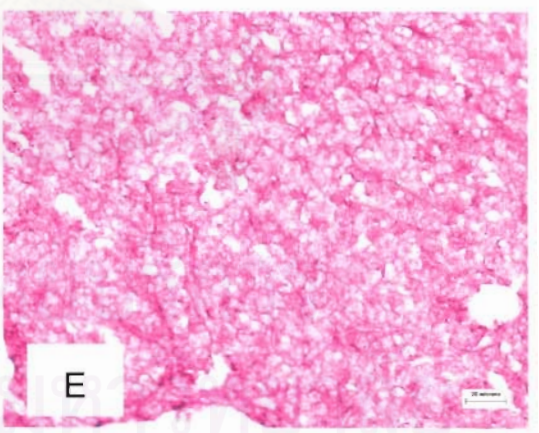
Figure G Photomicrograph of the 1 ppb endosulfan treated liver shows more positive PAS staining of glycogen deposition in liver tissue when compare to control liver.

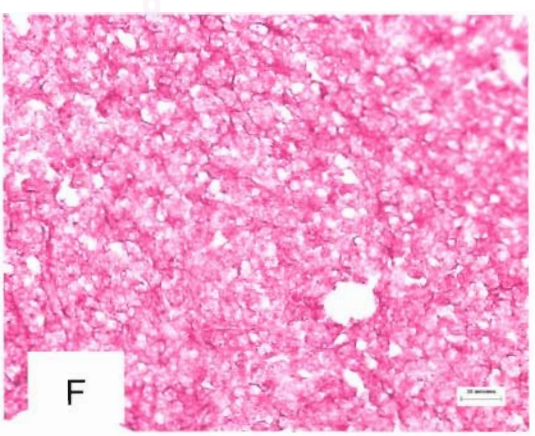

A

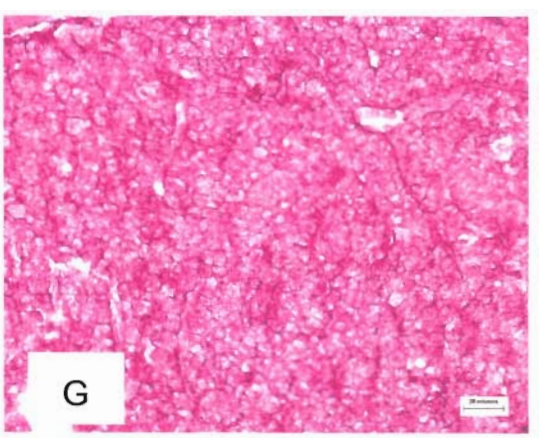

B


C


D


E


F


G

Gas Chromatography study

The result of the physical factor

Dissolved oxygen (DO) is necessary for aquatic life, and most state water quality regulations include a standard for dissolved oxygen, the typical value above of DO is 4 or 5 mg/l. In the study, DO values were between 3.52 and 6.26 mg/l. Temperature were obtained between 28.9°C and 32.8°C. Finally, pH at Klong 7 is close to the neutral value at 6.0 to 7.0. The water quality in Klong 7 was in good condition for aquatic animals survival (Table 4.5).

Table 4.5 DO, temperature and pH of water at klong 7 during March to November 2004

Month	DO (mg/l)	Temperature (°C)	pH
March	6.26±0.46	32.5±0.3	6.5±0.4
April	6.23±0.26	32.8±0.3	6.5±0.2
May	4.00±0.18	30.7±4.0	6.0±0.1
June	4.17±0.27	30.4±0.3	6.0±0.2
July	3.53±0.26	31.3±0.1	7.0±0.2
August	3.57±0.27	31.1±0.3	6.5±0.1
September	3.52±0.26	28.9±0.1	6.5±0.6
October	4.08±0.18	30.8±0.1	6.5±0.7
November	5.73±0.46	31.3±0.4	6.5±0.1

The result of calibration curve

The mixed standard solution concentrations of each organophosphate insecticide 1, 2, 5, 8 and 10 mg/l were plotted against peak heights. The result showed linear relationship, indicate by R^2 .The correlation coefficient was obtained at 0.96715 –

0.99573. The calibration curve was shown in APPENDIX D. The correlation coefficient of each organophosphate insecticide was shown in Table 4.6

Table 4.6 Correlation coefficient (R^2) of each insecticide in mixed organophosphate insecticides.

No.	Insecticides	Correlation coefficient (R^2)
1.	o,o,o-Triethylphosphothioate	0.981
2.	Thionazin	0.978
3.	Sulfotepp	0.995
4.	Dimethoate	0.961
5.	Disulfoton	0.985
6.	Methyl parathion	0.975
7.	Ethyl parathion	0.979
8.	Famphur	0.977

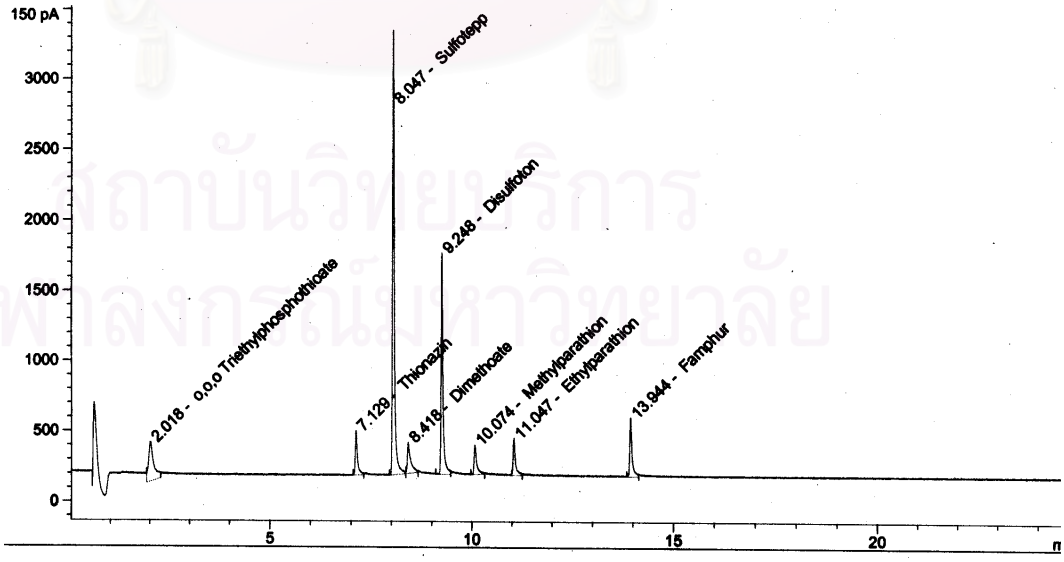
The result of the retention time

The retention time of each organophosphate insecticide was found by comparison with its retention time of a reference chromatogram. Between the retention time of 2.018 -13.944 min, the elution order of insecticides was o,o,o-Triethylphosphothioate, Thionazin, Sulfotepp, Dimethoate, Disulfoton, Methyl parathion, Ethyl parathion and Famphur, respectively. The retention time and elution order in this study were shown in table 4.9 and chromatogram of retention time was showed in Figure 4.1

Table 4.7 The elution order and retention time of each organophosphate insecticide

No.	Pesticides	Retention time (min)
1.	o,o,o-Triethylphosphothioate	2.01
2.	Thionazin	7.12
3.	Sulfotepp	8.04
4.	Dimethoate	8.41
5.	Disulfoton	9.24
6.	Methyl parathion	10.07
7.	Ethyl parathion	11.04
8.	Famphur	13.94

Figure 4.1 The chromatogram of the retention time of organophosphate insecticides (10 ppm)



The result of Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were defined by the peak height of analyte mix standard solutions that signal significantly different from the peak height of noise equal 3 for LOD and 10 LOQ of each compound. The LOD of organophosphate insecticides obtained were 0.002 to 0.050 mg/l (ppm) and LOQ were 0.007 to 0.196 mg/l (ppm). The result of LOD and LOQ of organophosphate insecticides was showed in Table 4.8

Table 4.8 LOD and LOQ of each organophosphate insecticide

No.	Pesticides	LOD (mg/l)	LOQ (mg/l)
1.	o,o,o-Triethylphosphothioate	0.020	0.060
2.	Thionazin	0.050	0.196
3.	Sulfotepp	0.002	0.007
4.	Dimethoate	0.040	0.135
5.	Disulfoton	0.009	0.031
6.	Methyl parathion	0.020	0.065
7.	Ethyl parathion	0.011	0.037
8.	Famphur	0.020	0.076

The result of % recovery and percent relative standard deviation (%RSD)

Percent of recovery is assessing to find the accuracy and the efficiency of extraction. The recovery of matrix standard within the limits of 70 to 120 percent was acceptable. The percent of recovery in this study was 82 to 160 percent. Sulfotepp and Famphur were over standard limit. The percent recovery of organophosphate insecticides and relative standard deviation were shown in Table 4.9.

% RSD is assessing in order to understand the precision of the method; low % RSD is defined as good precision. This study, %RSD values were about 4.65 to 29.66 percent, which is acceptable. The %RSD was shown in Table 4.9.

Table 4.9 Percent recovery of organophosphate insecticides and relative standard deviation (%RSD) 10 mg/l (ppm).

No.	Insecticides	% Recovery					Mean	SD	% RSD
		1	2	3	4	5			
1.	o,o,o-Triethylphosphothioate	79.0	85.0	103.0	82.0	82.0	86.2	9.62	11.16
2.	Thionazin	108.5	108.0	118.2	108.2	100.5	108.4	5.05	4.65
3.	Sulfotepp	95.0	88.0	160.0	160.0	102.0	121.0	35.90	29.66
4.	Dimethoate	72.0	80.2	76.1	92.1	82.3	80.5	5.57	6.91
5.	Disulfoton	89.0	105.0	98.2	115.0	113.0	104.0	10.7	10.2
6.	Methyl parathion	112.0	94.8	93.5	95.0	89.0	96.8	8.80	9.09
7.	Ethyl parathion	99.0	111.2	105.1	103.7	95.1	102.8	6.13	5.96
8.	Famphur	104.0	105.0	160.0	160.0	96.0	123.1	33.9	27.53

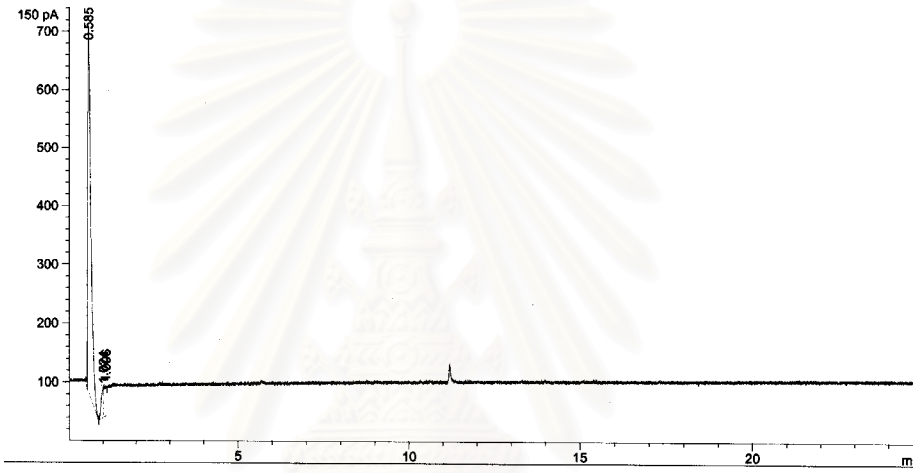
Quality Control

Blank

Sample blank

The organophosphate insecticides in the sample blank was non-detectable, indicated that there was no contamination. The chromatogram of sample blank was shown in Figure 4.2

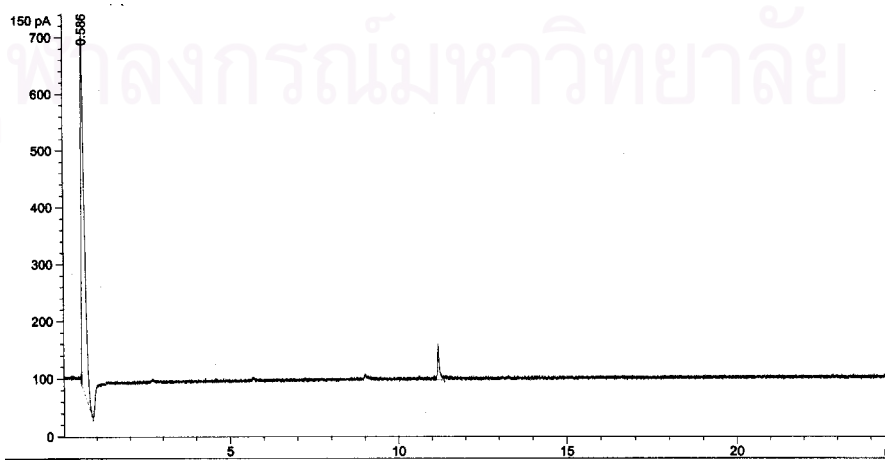
Figure 4.2 chromatogram of sample blank



Solvent blank

The solvent blank was non-detectable indicated that there was no contamination organophosphate insecticides contaminate. The chromatogram of solvent blank shown in Figure 4.3

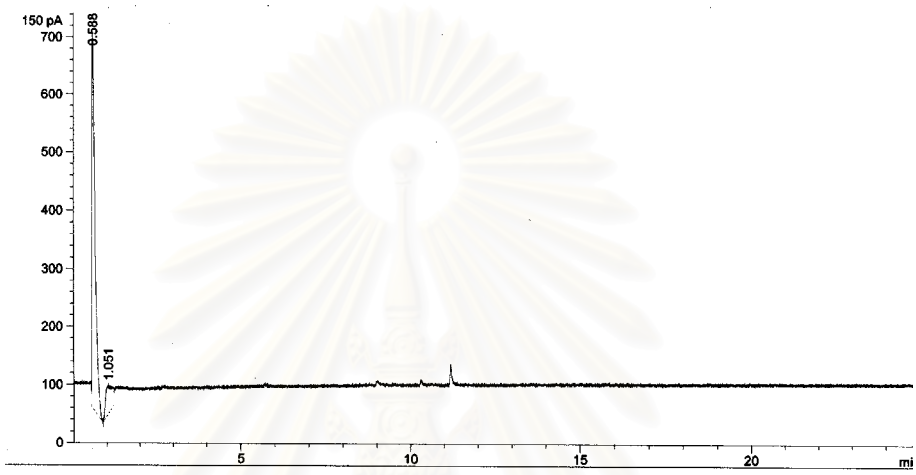
Figure 4.3 chromatogram of solvent blank



System blank

The system blank was non-detectable indicated that there was no contamination organophosphate insecticides contaminate. The chromatogram of solvent blank shown in Figure 4.4

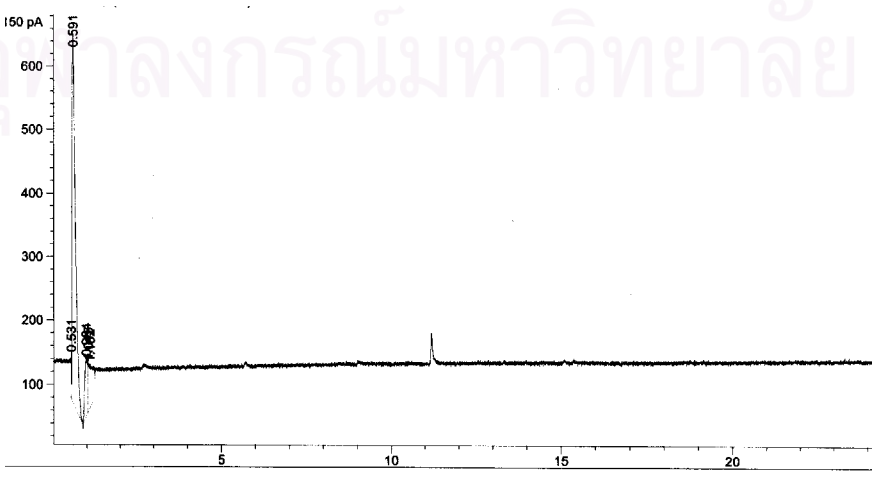
Figure 4.4 chromatogram of system blank



The result of organophosphate insecticide residuals at klong 7

The organophosphate insecticides residue was analyzed in water of klong 7 during March to December 2004. In all sites along the distance of Klong 7, the organophosphate insecticides residue was non-detectable. The example of chromatogram of organophosphate insecticide residues was shown in Figure 4.4 Other chromatograms were showed in APPENDIX D.

Figure 4.5 Chromatogram of organophosphate insecticides residual at klong 7



CHARPTER V

DISCUSSION

Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organ (Hinton and Lauren, 1990). The use of hepatic histopathological biomarkers has been recommended for biomonitoring (Hinton and Lauren, 1990). The liver has a primary role in the metabolism and excretion of xenobiotic compounds in digestion and storing and also in the production of yolk protein. The structural alteration can obviously occur in some toxic conditions. Therefore, the structure of fish liver is an integrator of biochemical and physiological function, when altered by previous exposition to toxicants, can produce biomarkers (Hinton and Lauren, 1990).

Gross anatomy of the Common Silver Barb liver *Puntius gonionotus*

Klong 7 fish and endosulfan treated fish that exposed to endosulfan concentration level of 1 ppb. The gross anatomy of the liver should that the liver color was light yellow, this is different from the control fish and it is in agreement with Robert (1978) who demonstrated that the liver of fish cultured in fish farm has lighter liver color than liver of fish in the nature. The liver structure of the Common Silver Barb is not clearly separated lobes and contact along the digestive tract, which are different from catfish (hybrid of *Clarias gariepinus* and *C. macrocepharus*) that the liver has one lobe and polygonal structure (Wattanasirmkit, 1993). The liver structure of the Common Silver Barb composed of hepatocytes arranged between the sinusoid and was similar to the liver structure of the channel catfish (Hinton and Pool, 1976).

Moreover, Klong 7 fish and endosulfan treated group have the liver that becoming soft and white spots throughout the liver. This symptom may be occurring inflammatory (Hinton and Pool, 1976). Normally, the relative liver weight (%RL) of the common fish should be 1 – 2 % (Roberts, 1987). In this study all the ratios were normal compared to the standard ratio. The relative liver weight of Klong 7 fish and endosulfan

treated fish at all concentration levels were significantly less than the control group ($p \leq 0.05$). Acute inflammation increased liver weight because of the hepatocytes swelling which is the same as endosulfan treated fish liver in this study. On the other hand, subchronic inflammation decreased liver weight because of the karyolysis and cell necrosis, similarly to Klong 7 fish liver. The body weight of treated endosulfan fish decreased as same as the study of lindane exposed on rats (Ramalingsm et al., 2001). This effect was similar to the study of Georgem (1982) who reported about acute and chronic toxicity of monochlorobenzene on rainbow trout that reduced body weight.

Histopathological change of the Common Silver Barb liver at Klong 7

The common hepatic injury of the Klong 7 fish in dry season was more severe than in rainy season. It was found that slightly hydropic swelling and blood congestion were similar to the effect of sublethal toxicity of $CdCl_2$ (Rani and Ramamurthi, 1989). TBTO treated liver of rainbow trout also revealed swollen hepatic cell (Schwaiger et al., 1992). The generative change was hydropic swelling which was reported in case of severe intoxication (Wattanasirmkit and Patamastan, 1997). Pyknotic and karyolysis of hepatocytes, damaged blood vessels in parenchyma were also the same as Sakthivel and Gaikward (2002) who studied on *Gambusia affinis* that exposed to dimecron (organophosphate insecticide). The foci necrosis of hepatocytes was noticed. Roberts (1978) reported that foci necrosis is commonly found in fish liver. In addition, foci hepatic necrosis is a regular lesion caused by the virus disease of salmonoids and channel catfish. Pericentral necrosis has been reported in trout and catfish that received relatively high dose of CCl_4 or MBC (Gaingerich et al., 1977; Gingerich and Dalich, 1987). The effect of fenvalerate on liver of *Ctnopharyngodon idellus* was reported about tissue damages like necrosis (Tilak et al., 2001). Our studies were similar to the result of Kumar and Pant (1981) on the effect of copper and zinc on liver of *Puntius conchonis* that showed hepatocytes necrosis. In trout, foci necrosis was evident by nuclear lysis, pyknotic nucleus, increase cytoplasmic eosinophilic (Droy, 1988). The diffuse necrosis in this study was found in a few cases and the majority were found at the margin area of liver tissue. The diffuse necrosis was commonly observed in CCl_4 acute toxicity studies

and subcapsule necrosis was also found (Gingerich et al., 1978) which is the same as the effect of nickel on lake whitefish (Ptashynski et al., 2002). Moreover, in this study, hyalin droplet deposition, dilation of sinusoid and lipid accumulation were found. All these damages were similar to the result of Gerundo (1991) who studied the liver of rainbow trout exposed to malachite green. Patward and Gaikard (1991) reported on the effect of insecticides "sumithion" on the liver of *Gambusia affinis affinis* which is similar to the result of Waster (1990) who reported on the hepatotoxicity of Guppy *Peocilia reticulata* of di-n-butyltin dichloride and Fanta (2001) who studied sublethal effect of folidol on *Corydoras paleatus* that caused hepatocytes injury. The inflammatory reaction in the liver indicated by rupture of endothelium of sinusoid, central vein, bile duct, pancreas and capsule of liver. Moreover, hepatic degeneration and perivenular fibrosis were detected. In addition, hemorrhagic inflammation of which there were many erythrocytes in the area of damaged tissue and the invasion of macrophage and leucocytes in liver parenchyma was found. These results were the same as the effect of arsenic exposure in lake whitefish *Coregonus clupeaformis* and the impact of TCDD on rainbow trout liver (Gail et al., 2000). The finding of fibroplasias indicated the regeneration of the liver tissue. The chronic hepatic inflammation was characterized by the replacement of parenchyma with new fibrous connective tissue (Rand and Petrocelli, 1985). In addition, in this studies abnormality of red blood cells with the swelling, without the cytoplasm and occlusion were the same as the report on chlorine toxicity on *Oreochromis massmbicus* (Ramalingam and Murabai, 2002). These results were similar to the effect of dimicron on *Heteropneustes fossilis* (Anand et al., 2001).

Not only the liver injury but also the bile duct and pancreas in liver parenchyma were damaged. The bile duct was distorted with pancreas inflammation, blood congested in pancreas, pancreas shrinkage, infiltration of macrophage and leucocytes and lost of zymogen. Ptashynski (2002) also reported about the lake whitefish contaminated by nickel the result was similar to the effect of folidol that cause damage of bile duct and pancreas to *Corydoras paleatu* (Fanta et al., 2001).

Hepatic cell damages in large body size and small body size of Klong 7 fish were not different. Both dry and rainy season showed irreversible hepatocytes injury. Moreover, reversible damage; sinusoid dilates, cell swelling and blood congestion were often found. Therefore, the body size of Klong 7 fish has no effect on the lesion level of hepatocyte injuries (APPENDIX C).

Although in this study, the organophosphate insecticide residues in the water at klong 7, Pathumtani province was undetected, the organochlorines insecticide residues can be detected. The trend of the organochlorine insecticide residues was observed in rainy season with more concentration level than dry season. Therefore, the organochlorine insecticide can have effect on fish liver. The Klong 7 fish exhibited more severe lesion on liver than endosulfan treated fish can be because Klong 7 water had many insecticides contaminated. Therefore, their toxicants were synergistic and more toxic to fish liver.

Lipid and glycogen accumulation in liver of the Common Silver Barb at Klong 7

Glucose cannot accumulate in large quantity inside the cells because the accumulation of charged glucose-6-P can cause cells to swell. Instead, glucose is stored in the form of non-charged or in the form of glycogen. The normal glycogen content of liver varies between 2 and 8%. The pathways of glycogen controlled by the glycogen phosphorylase are homodimeric enzymes. The enzyme is also subject to covalent modification by phosphorylation as a means of regulating its activity. The relative activity of the un-modified phosphorylase enzyme is sufficient to generate enough glucose-1-phosphate for entry into glycolysis for the production of sufficient ATP to maintain the normal resting activity of the cell. This is true in both liver and muscle cells.

One might predict that the pathway for the synthesis of fatty acids would be the reversal of the oxidation pathway. The pathway for fatty acid synthesis occurs in the cytoplasm, whereas, oxidation occurs in the mitochondria. The other major difference is

the use of nucleotide co-factors. Oxidation of fats involves the reduction of $FADH^+$ and NAD^+. Synthesis of fats involves the oxidation of NADPH. However, the essential chemistry of the two processes are reversals of each other. Both oxidation and synthesis of fats utilize an activated two carbon intermediate, acetyl-CoA. However, the acetyl-CoA in fat synthesis exists temporarily bound to the enzyme complex as malonyl-CoA. The synthesis of malonyl-CoA is the first committed step of fatty acid synthesis and the enzyme that catalyzes this reaction, acetyl-CoA carboxylase (ACC), is the major site of regulation of fatty acid synthesis.

The liver has a central function in maintaining homeostasis of the organism by synthesis and secrete of molecules into the blood as well as by removal, metabolism and eventually excretion of compounds for example this organ governs blood glucose level and accumulation of lipid in tissue (Daniel, 2001). The results of histochemistry observation in fish liver at Klong 7, both dry season and rain season, were the decrease of glycogen accumulation in liver. The result of zinc exposed to the liver of Channa puntatus was the significant reduce of glycogen in liver (Srivastava et al*.,* 2002). The impact of fenvalerate on fish *Ctenopharyngodon idellus* decreased level of glycogen (Tilak and Yacobu, 2002). The effect of cadmium chloride on marine edible gastropod changed glycogen level (Khan *et a.,* 2001). Rainbow trout exposed to TCDD reduced glycogen level in liver tissue (Gail et al*.,* 2000). Lipid accumulation in liver tissue in this study decreased comparing to the control liver. The amount of lipid droplets different between dry season and rainy season, In dry season the larger size of lipid droplets was found but less in number than in rainy season. This result was the same as the effect of zinc exposed to liver of *Channa puntatus* (Srivastava et al., 2002). These results were similar to the lipid accumulate in the toxicity of industrial pollution on *Liza parsia* that decreased lipid accumulation (Bharatha et al., 2001). Hymavathi and Roa (2001) reported the effect of pollution that decreased lipid deposition in organism. Desai (1984) also reported about *Tilapia mossambica* contaminated to monocrotophos and showed fatty degenerated.

Histopathological changes of Common Silver Barb liver after exposed endosulfan

The endosulfan is an organochlorine insecticide. It is practically water insoluble, but readily adheres to clay particles and persists in soil and water. This compound is extremely toxic to most fish (Syed and Chetana, 2004).

Histological alterations detected in liver tissue of Common Silver Barb were endothelium of central vein and capsule ruptured, blood congested, cell necrosis, hyalin droplet accumulation, pyknotic nucleus and karyolysis. Moreover, red blood cell was swelling, bile duct and pancreas inflammatory and infiltration of macrophage were detected. The cell injury was similar to the result of endosulfan exposed on liver of *Heteropneustes fossilis* that showed the damage of hepatic cells (Rawat et al., 2002). The cytological effects after exposed to endosulfan on rainbow trout (Baglio and Farber, 1965) showed the immigration of macrophages like the finding in this experiment.

Lipid accumulation studied by frozen tissue and Oil red O staining technique the showed the depletion of lipid droplet accumulation in liver in all concentrations when compared to the control group. The sublethal effect of atrazine and dichlobenil on rainbow trout reflected in an increase in the total lipid in liver (Elesovic et al., 1997). Very little change was observed on lipid droplets accumulation in hepatocytes after contaminated with endosulfan on carp (Gas and Serfaty, 1972). Glycogen deposition in liver after contaminated with endosulfan displayed more glycogen in hepatocytes than the control group in all concentrations. This result was the similar to the chloroantiline that contaminated the rainbow trout (Hacking et al., 1978). Radhaiah et al. (1987) reported that heptachlor is organochlorines, which induced an increase of glycogen in liver of *Tilapia mossambica*. On the contrary, glycogen stores were depleted after exposure to endosulfan on rainbow trout (Baglio and Farber, 1965). The liver of carp fed with endosulfan-contaminated food was investigated about glycogen depletion in rainbow trout that paralleled to carp (Hacking et al., 1978). On the other hand, Silberberg (1974) who studied the effect of dieldrin on fish and the significant decrease glycogen in liver were reported. The lipid and glycogen accumulated in hepatocytes of endosulfan treated fish in this study was different from Klong 7 fish because it was

contaminated with endosulfan for shorter period than Klong 7 fish, therefore the metabolism of lipid and glycogen in cell was different.

Gas chromatography Analysis (GC)

Organophosphate insecticide residues in the water of Klong 7 were non-detectable because the organophosphate can be rapidly degraded in the environment. The half-life of disulfoton in water ranges from 5 hours during summer to 12 hours during winter. Methyl parathion can be degraded by direct photolysis in natural water with half-life of 8 days in summer and 38 days in winter. Moreover, the organophosphates could be transformed into other compounds or other structures, for example methyl parathion can be rapidly transformed to paraoxon, the vapor will be rapidly photolyzed, half-life of 5 min in summer. The parathion residue on foliage will decay with a half-life of 1 day reaching low levels in a week or two. It will bind tightly to soil and decay by biological and chemical hydrolysis in several weeks, forming p-nitrophenol, diethylthiophosphoric acid, and paraoxon (Fanta et al., 2001). Then, GC cannot detect the new compound because the new compound was changed in structure and property that affects on the retention time of the compound. Another reason is probably because the farmers in this agricultural area used the organophosphate insecticides less than other compounds, therefore organophosphate insecticide residues in water were too low. LOD and LOQ values were lower than the maximum residue limit in water (WHO, 1992). Therefore, the amount of organophosphate insecticide residues in Rangsit Klong7 area the present is acceptable. In the future, if the uses of organophosphates increase rapidly, organophosphate insecticides may cause harmful effects to organisms in the environment. Therefore, the monitoring of organophosphate insecticide residues must always be carried out in nature.

CHARPTER VI

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. % Relative liver weight (%RL) of Klong 7 fish and all concentrations of endosulfan treated fish were significant by lower than the control fish ($P \leq 0.05$)
2. The liver of the Common Silver Barb *Puntius gonionotus* responded with cell injuries to contaminated water in Klong 7.
3. The Klong 7 fish *Puntius gonionotus* liver was severely damaged in dry season more than in rainy season.
4. Histochemical studies of lipid and glycogen accumulation in Klong 7 liver showed the decrease in glycogen accumulation.
5. Histopathological alteration of endosulfan treated liver was dose dependent effect.
6. Histochemical studies of lipid and glycogen accumulation in endosu ed liver showed the increase of glycogen accumulation but decre: ›id accumulation in liver.
7. The studies of organophosphate insecticide residues in water at Klong 7 by GC/FPD showed negative result.

Recommendations

1. The studies on the subchronic test in the laboratory should be set up for other insecticide residues.
2. More organophosphate insecticide standards species should be added to determine other types of residues by GC/FPD.

REFERENCES

Adachi, Y., Bradford, B. U., Gao, W., Bojes, H. K., and Thurman, R. G. 1994. Inactivation of Kupffer cells prevents early alcohol-induced liver injury. Hepatology 20: 453-460.

Alex V. S., Jonathan T. H., Swee J. T. and David E. H. 2000. Thiobencarb-induced embryotoxicity in medaka (*Oryzias latipes*): stage-specific toxicity and the protective role of chorion. Aquatic Toxicology 48: 309-326.

Alexandra, L., and Alice, H. 2004. A comparative assessment of the adrenotoxic effects of cadmium in two teleost species, rainbow trout, *Oncorhynchus mykiss*, and yellow perch, *Perca flavescens*. Aquatic Toxicology 67: 13-21.

American Society for Testing and Materials. 1980. Standard practice for conducting toxicity tests with fishes, macroinvertebrates, and amphibians. ASTM E 729-80, Philadelphia: ASTM.

Anand, K. A., Tripathy, A. P., and Tripathy, N. K. 2001. Effect of dimecron on the blood parameters of *Heteropneustes fossilis*. Journal of Environmental Biology. 22: 297-299.

Anat, T., and Paul, F. H. 1998. Pesticides use and residual occurrence in Thailand. Department of Geography and environmental Science Program: University of North Taxas, Denton, U.S.A.

Anderson, B. S., Hunt, J. W., Phillips, B. M., Nicely, P. A., Vlaming, V., Connor, C., Richard, N. and Tjeerdema, R. S. 2003. Integrated assessment of the impacts of agricultural drainwater in the Salinas River (California, USA). Envronmental Pollution 124: 523-532.

Anderson, W. A. D. and Kissane, J. M. 1977. Pathology. Missouri: Mosby company.

Arapoglou, D., Vlyssides, A., Israilides, C., Zorpas, A. and Karlis, P. 2003. Detoxification of methyl-parathion pesticide in aqueous solutions by electrochemical oxidation. Journal of Hazardous Materials B97: 191-199.

Aspelin, A. L., Grube, A. H. 1998. Pesticide Industry Sales and Usage: 1996 and 1997 Market Estimates. Office of Prevention, Pesticides and Toxic Substances, U. S. Environmental Protection Agency. Washington: DC. 37 pp.

Asoprim and Avans. 2001. The effect of pronephros on histology and ultrastructure and activity of immunocompetent polymorphonuclear and mononuclear cell in European Catfish (*Silurus glanis*). Aquatic Toxicology 66: 15 -32.

Balasubramanian, P., Saravanan, T. S., and Palaniappan, M. K. 1998. Biochemical and histopathological changes in certain tissue of *Oreochromis mossambicus* (Trewaves) under ambient urea stress. Bull. Environ. Contam. Toxicol. 63: 117–124.

Barbara Nowak. 1992. Histological changes in gills induced by residues of endosulfan. Aquatic Toxcicology 23:65-83.

Bautrup, E., Gissel, M., and Danschner, G. 1986. Histochemical demonstration of two mercury pools in trout tissue : mercury in kidney and liver after mercuric chloride exposure. Ecotoxicology and Environmental Safety 12: 267-282.

Bender, B. 1966. Toxicity of pesticides. San Francisco: Freeman.

Biagianti-Risbourg, S. and Bastide, J. 2000. Hepatic perturbations induced by a herbicide (atrazine) in juvenile grey mullet *Liza ramada* (Mugilidae, Teleostei): an ultrastructural study. Aquatic Toxicology 31: 217-229.

Birgul, O., Elif, I. C., Zeki, M. Y., Ozkan, U. and Erhan, U. 2004. The effects of endosulfan on the great ramshorn snail *Planorbarius corneus* (Gastropoda, Pulmonata): a histopathological study. Chemosphere.

Bharatha, L. B., Rao K. M., and Bangaramma, Y. 2001. Impact of indrustrial pollution on the nutritive value of *Liza parsia* from harbour waters of Vizag. Aquacult 2: 111-116.

Bharathi, C., Sandeep, B. V., and Subbarao, B. 2002. Effect of monocrotophos on the oxygen consumption of a freshwater edible crab. Paratelphusa hydrodromus. J. Ecophysio Occupl Hlth 2: 85-88.

Borges, G., Mendonca, P., Joaquim, N., Coucelo, J., and Aureliano, M. 2003. Acute effects of vanadate oligomers on heart, kidney, and liver histology in the Lusitanian toadfish (*Halobactrachus didatylus*). Arch. Environ. Contam. Toxicol. 45: 415-422.

Bradford, B. U., Misra, U. K., and Thurman, R. G. 1993. Kupffer cells are requiured for the swift increase in alcoholic metabolism. Res. Common. Subst. Abuse. 14: 1-6.

Braun, T. and Volkl, A. 1991. Toxicant induced cytological alterations in fish liver as biomarkers of environmental pollution: A case study on hepatocytocellular effects of dinitro-o-cresol in golden ide (*Leuciscus idus melanotus*). Fish in ecotoxicology: 55-80.

Carvalho, F. P., Gonzalez, F. F., Villeneuve, J. p., Cattini, C., Hernandez, G. M. and Mee, L. D. 2002. Distribution, Fate and effects of pesticide residues in tropical coastal lagoons of northwestern Mexico. Environ Technol. 23:1257-1270.

Casteleijn, E., Kuiper, J., van Rocij, H. C. J., Kamps, J. A. A. M., Koster, J. F., anddd Van Berkel, T. J. C. 1988. Homonal contral of glycogeonlysis in parenchymal liver cells by Kupffer and endothelial liver cells. In: Thurmam, R. G. 1998. Machanisms of hepatic oxicity. II. Alcoholic liver injury involved activation of Kupffer cells by endotoxin. Am. J. Physiol. 275(38): G605-G611.

Chan, S., Chhuon K. C., Bouakhamvongsa, K., Suntornratana, U., Yoorong, N. 1999. Fish migrations and spawning habits in the Mekong mainstream. Assessment of Mekong fisheries: Fish Migrations and Spawning and the Impact of Water Management Project (AMFC). AMFP Report 2/99. Vientiane, Lao, P.D.R.

Chan, S. 1999. Freshwater Fish. Bangkok: Odientstore.

Chumraskul, P. and Sakulteangtong, S. 1999. Diatribution of pesticides in water and sediments in Mae Klong River and Tributaries. Toxic substances news and report 26(2): 43-70.

Chumraskul, P. and Sima, B. 1997. Organophosphorus and carbamates insecticide residues in water and sediments from Ta-Jean River and Tributaries. Toxic substances news and report 24(2): 55-64.

Chumraskul, P. and Sima, B. 1997. Organophosphorus and carbamates insecticide residues in water and sediments from Bang-Pa-Kong River and Tributaries. Toxic substances news and report 23(3): 101-112.

Chumraskul, P. and Sima, B. 1997. Organophosphorus and carbamates insecticide residues in water and sediments from Pa-Sak River and Tributaries. Toxic substances news and report 24(3): 40 -52.

Cooley, H. M., Evans, R. E., and Klaverkamp, J. F. 2000. Toxicological effects of dietary uranium in lake whitefish (*Coregonu clupeaformis*). Aquatic Toxicology 48: 495 -515.

Crabb, D. W., Qulali, M., and Dipple, K. M. 1991. Endrocrine regulation and methylation patterns of rat class I dehydrogenase in liver and kidney. Adv. Exp. Mol. Biol. 284: 277-284.

Culling, C. F. A. 1963. Handbook of histopathological technique. 2nd ed. London: Butter worth.

Dalene, V. H., Andre, V., and Mikko N. 2004. Effects of short-term copper exposure on gill structure, metallothionein and hypoxia-inducible factor-1α (HIF-1α) levels in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology 69: 271-280.

Daniel, S. and William, H. B. 2001. Target organ toxicity in marine and freshwater teleosts. Taylor & Francis Press, New York, U.S.A. 372 pp.

Das, B. K., and Mukherjee, S.C. 2000. Chronic toxic effects of quinalphos on some biochemical parameters in *Labeo rohita* (Ham.). Toxicology Letter 114: 11-18.

David, J. S., William, H. G. and Maria, G. 1999. Xenobiotic in fish. Plenum Publisher, New York, p 223.

David, E. H. 1993. Toxicologic histopathology of fishes: A systemic approach and overview. CRC Press, London. 552 pp.

Davidson, A. 1975. Fish and fish dishes of Laos. Imprimerie Nationale Vientiane. 202 p.

Decker, T., Lohman-Matthes, M. L., Karch, U., Petrs, T., and Decker, K. 1989. Comparative study of cytotoxicity, tumor necrosis factor and prostaglandin release after stimulation of rats Kupffer cells, murine Kupffer cells and murine inflammatory liver macropharges. J. Leukoc. Biol. 45: 139-146.

Desai, H. S., Nanda B., Panigrahi J. 2002. Toxicological effects on some biochemical parameters of fresh water fish *Channa punctatus* (Bloch.) under the stress of nickel. Journal of Environmental Biology. 23: 275-277

Desai, A. K., Joshi, U. M., and Ambadkar, P. M. 1984. Histological observations on the liver of *Tilapia mossambica* after exposure to monocrotophos, an organophosphorus insecticides. Aquatic Toxicology 21: 325-331.

Department of agriculture (DOA). 1991. Statistics of imported pesticides to Thailand, Bangkok :Misnistry of Agricultural and Cooperatives.

Department of agriculture (DOA). 1996. Statistics of imported pesticides to Thailand, Bangkok :Misnistry of Agricultural and Cooperatives.

Department of agriculture (DOA). 2003. Statistics of imported pesticides to Thailand, Bangkok :Misnistry of Agricultural and Cooperatives.

Dey, M., and Gupta, A. 2002. Acute toxicity of endosulfan on three anuran tadpoles. J Ecotoxico. Environ. Monit. 12: 61-65.

Dhembare, A. J., Sonawane N. V., and Gacche, R. N. 2003. Haematological responses in *Rattus norvegicus* Linn. exposed to malathion. J. Expt. Zoo India, 6: 117-119.

Di Giulio, R. T. 1991. Indices of oxidative stress as biomarkers for environmental contamination. In: Cooley, H. M., Evans, R. E., and Klaverkamp, J. F. 2000. Toxicological effects of dietary uranium in lake whitefish (*Coregonu clupeaformis*). Aquatic Toxicology 48: 495 -515.

Diwakar, M., Sunil, K. S. and Ajai, K. S. 2003. Effects of the insecticide cypermethrin on plasma calcium and ultimobranchial gland of a teleost, *Heteropneustes fossilis*. Ecotoxicology and Environmental Safety: Article in Press, Corrected Proof.

Duangswasdi, S. and Pupipat, T. 1982. Summary on life history of introduced of fish Species. Technical paper NO7/1892. National Inland Fisheries Division, Department of Fisheries.

Eduardo and Rogerio. 1999. Comparative Histalogy. London: Academic Press.

Edward, D. L., Holly, A. S, Sue, D., and Elwood L. Developmental chlorpyrifos effects on hatchling zebrafish swimming behavior. Neurotoxicology and Teratology: Article in Press, Corrected Proof.

Fanta, E., Rios, F. S., Romao, S., Vianna, A. C. and Freiberger, S. 2003. Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. Ecotoxicol. Environ. Saf. 54: 119-130.

Fernández-Vega, C., Sancho, E., Ferrando, M. D., and Andreu E. 2002. Thiobencarb-Induced Changes in Acetylcholinesterase Activity of the Fish *Anguilla anguilla*. Pesticide Biochemistry and Physiology 72: 55-63.

Fishbein, F. 1974. Histological studies on the effects of PCB to liver, kidney of Catfish (*Clarias gariepinus*) . Aquatic Toxicology 52: 255-271.

Folmar, L. C., Gardner, G. R., Hickey, J., Bonomelli, S., and Moody, T. 1993. Serum chemistry and histopathological evaluations of brown bullheads (*Ameiurus nebulosus*) from the Buffalo and Niagara rivers, New York. Arch. Environ. Contam. Toxicol. 25: 298-303.

Fuad, A. T., Hellmut, M., Oliver, T., and Thomas, D. 2001. An analytical method for the rapid screening of organophosphate pesticides in human biological samples and foodstuffs. Forensic Science International 121: 126-133.

Ilarria, C., Michela, M., Cristiana, S., Lucia, L. and Silvano, F. 2003. Fish as bioindicators of brackish ecosystem health: integrating biomarker responses and target pollutant concentrations. Oceanologica Acta 26: 129-138.

Gail, L. W., Paul, D. J., and John, P. G. 2000. Pathologic alterations in adult rainbow trout, *Oncorhynchus mykiss,*exposed to dietary 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Aquatic Toxicology 50: 287-299.

Gerundo, N., Alderman, D. J., Clifton-Hadley, R. S., and Feish, S.W. 1991. Pathological effects of repeated doses of malachite green. A preliminary study. J. Fish Dis. 14(5): 521-532.

George, M. D., Robert, E. L., and William, H. G. 1982. Acute and chronic toxicity studies with monochlorobenzene in rainbow trout. Aquatic Toxicology 2: 127-142.

George, S. 1999. The Aquatic environment. Fresno: Thomson Publications.

Gimeno, A., Ferrando, M. D., Sanchez, S., and Andreu, E. 1994. Endosulfan effects on liver and blood of the eel, *Anguilla anuilla*. Comparative Biochemmistry and Physiology Part C: Phamacology 108: 343-348.

Gingerich, W. P. 1977. Histopathology of fishes. Aquatic Toxicology Vol.1

Ghosh, A. R. and Chakrabarti, P. 1993. Histopathlogical and histochemical changs in liver, pancreas and kidney of the freshwater fish *Heteropnuestes fossilis* bloch exposed to cadmium. Environment and Ecology 11(1): 185-188.

Ghousia Begum. 2004. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (linn) and recovery response. Aquatic Toxicology 66: 83.

Goncaleves, C., and Alpendurada, M. F. 2002. Multiresidue method for the simultaneous determination of four groups of pesticides in ground and drinking waters, using solid-phase microextraction-gas chromatography with ECD and thermionic specific detection. Chromatography A 968: 177-190.

Gurr, G. T. 1963. Biological staining Method. 7th. ed. London: George T. Gurr Ltd.

Hacking, H. and David, J. 1978. Sublethal effects of exposure to polychlorinated biphenyls in Rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology 62: 55-69

Harder, W. and Sokolof, S. 1975. Anatomy of Fishes. Stuttgart.

Haider, S. S., Haider, S. J. 2002. Arsenic-initiated peroxidative damage in brain, liver and kidney of pregrant rats. Cheml. Environ. Res.11: 189-192.

Handy, H. and Penrice, K. 1993. The effects of prolong exposure to Mercuric chloride on Rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology 65: 385-397.

Helge, A., Hans, J. P. and Thomas, B. 1996. Sublethal effects of prolong exposure to Disulfoton in Rainbow trout (*Oncorhynchus mykiss*): Cytological alterations in the liver by a potent Acetylcholine esterase inhibitor. Ecotoxicology and Environmental safty. 34: 43-55.

Hide, J. and Schulte, E. 1978. separation of organophosphorus pesticides and their metabolites on glass capillary column. Chromatographia. 11-17.

Hinton, D. E. and Lauren, D. J. 1990a. Integrative histopatological approaches to detecting effects of environmental stress on fishes. In: Adams, S. M. (ed.), Biological indicators of stress in fish, pp. 51-66. Bethesdda: American fisheries society symposium.

Hinton, D. E. and Lauren, D. J. 1990b. Liver structural alterations accompanying chronic toxicity in fish: potential biomarkers of exposure: In Wu, W. Z., Zhou, B. S., Xu, Y., Schramm, K. W., and Kettrup, A. 1999. Cytological and biochemical alteration in *Carassius auratus* hepatocyte from exposure to sediment containing dioxin and related compounds. Arch. Environ. Contam. Toxicol. 37: 358-363.

Hinton, D. E. 1990. Histological techniques. Method in Fishes Biology. Marryland 43: 191-211.

Humason, G. L. 1979. Animal tissue technique. 4th ed. San Francisco: Freeman.

Hung, T., Michael, C., and Mehran, A. 2004. Method for the determination of organophosphate insecticides in water, sediment and biota. Chemosphere 54: 41-47.

Hymavathi, V., and Rao L. M. 2001. Effect of slaughter house pollution on the biochemical composition of *Channa orientalis*. Journal of Environmental Biology. 22: 209-212.

Iimuro, Y., Gullucci, R. M., Luster, M. I., and Thurman, R. G. 1997. Antibodies to tumor necrosis factor-α attenuate hepatic necrosis and inflammation due to chronic exposure to ethanol in the rat. Hepatology 26: 1530-1537.

Ingle, S. T., Shinde, D. N., and Namdas, S. B. 2003. Effects of rogar and endosulfan on the metabolism of fresh water sponge (*Spongilla lacustris*). Journal of Environmental Biology. 24: 63-67.

Jaffee, J. 1991. Pesticides Book. Philadelphia: W. B. Saunders.

Jau shin, S. 2003. Basic pathology. Boston: Massachusetts.

Jonsson, C. M. and Toledo, M. C. F. 1993. Acute toxicity of endosulfan to the fish *Hyphessobrycon bifasciatus* and *Brachydanio rerio*. Arch.Environ.Contam.Toxicol. 24: 151-155.

Jonh, P. J. and Prakash, A. 2003. Bioaccumulation of pesticides on some organs of freshwater Catfish *Mystus vittatus*. Bull. Environ. Contam. Toxicol. 70: 1013-1016.

Jyothi, B., and Narayan, G. 2001. Effect of pesticides carbaryl and phorate on serum cholesterol level in fish, *Clarias batrachus* (Linn). Journal of Environmental Biology. 22: 233-235.

Khan, A. K., Shaikh, A. M., and Anasari, N. T. 2001. Cadmium chloride toxicity in glycogen level from body part and whole body of marine edible gastropod *Babylonia spirata*. Uttar Pradesh J. Zoo 21: 203-206.

Khan, R. A. 2000. Comparison of tissue lesions in four species of benthic fish sampled in 1972–1973 and 1997–1998 on the Grand Bank off Newfoundland. Bull. Environ. Contam. Toxicol. 65: 78-83.

Khan, R. A. 2003. Stress-related bioindicator anomalies in feral male winter flounder (*Pleuroectes americanus*) exposed to effluent from two pulp and paper mills in Newfoundland. Bull. Environ. Contam. Toxicol. 70: 401-407.

Kotsanis, N. and Iliopoulou -Georgudaki, J. 1999. Arsenic induced liver hyperplasia and kidney fibrosis in rainbow trout (*Oncorhynchus mykiss*) by microinjection technique. Arch.Environ.Contam.Toxicol. 27: 151-155.

Kottelat, S. 1998. Freshwater Fish. Philadelphia: W. B. Saunders.

Kumar, V., Cotran, R. S., and Robbins, S. L. 2002. Robbins basic pathology. 7th ed. Philadelphia: W. B. Saunders.

Kumar, S. and Pant, S. C. 1984. Organal damage caused by aldicarb to a freshwater teleost *Barbus conchonius* Hamilton. Bull. Environ. Contam. Toxicol. 33: 50-55.

Kumuri, S. A., and Kumuri, N. S. 1995. Effect of water pollution on histology of fish *Channa Striatus* in Hussainsagar, Huderrabad, India. Environmental and Ecology 13: 932-934.

Kumuri, S. A., Sinsa, P. K., Gopal, K., and Prasad, K. 2001. Dietary intake of persistent organochlorine residues through Genetic fishes in India. Int. J. Eco Environ. Sci. 27: 117-120.

Lisa, N. T., Wendy J. M., Greg G. P., Patrice, C., and Gordon M. D. 2004. Use of performance indicators in evaluating chronic metal exposure in wild yellow perch (*Perca flavescens*). Aquatic Toxicology 67: 371-385.

Lliopoulou -Georgudaki, J. and Kotsanis, N. 2001. Toxic effects of cadmium and mercury in rainbow trout (*Oncorhynchus mykiss*) : A short-term bioassay. Bull. Environ. Contam. Toxicol. 66: 77-85.

Mantle, D., Saleem, M. A., Williams, F. M., Wilkins, R. M. and Shakoori, A. R. 1997. Effect of pirimiphos-methyl on proteolytic enzyme acxtivities in rat heart, kidney, brain and liver tissues in vivo. Clinica Chimica Acta 262: 89-97.

Mark, B., Mette, J., Erik B., and Mark, B. 2002. Exposure of juvenile guppies to three antiandrogens causes demasculinization and a reduced sperm count in adult males. Aquatic Toxicology 56: 227-239.

Mehmet, Y., Ali G., and Erhan K. 2004. Investigation of acute toxicity and the effect of cadmium chloride ($CdCl_2 \cdot H_2O$) metal salt on behavior of the guppy (*Poecilia reticulata*). Chemosphere 56: 375-380.

Michael, K., Maria, L. A., Tina, R., Richard F. and Carmel M. 2002. Rainbow trout primary epidermal cell proliferation as an indicator of aqutic toxicity: an in vitro/in vivo exposure comparison. Aquatic Toxicology 60: 43-59.

Michael, N., Matthias, L. and Ralf, S. 2003. A qualitative sampling method for monitoring water quality in temporary channels or point sources and its application to pesticide contamination. Chemposphere 51: 509-513.

Mishra, R. and Shukla, S. P. 1994. Effect of endosulfan on bioenergistic property of liver. Pesticides Biochemistry and Physiology 50: 240-246.

Moden, K., Arii, S., Itai, S., Sasaoki, T., Adachi, Y., Funali, N., Higashitsuji, H., and Tobe, T. 1991. Enhancement and hepatocyte modulating effect of chemical mediators

and monokines produced by hepatic macrophange in rats with induced sepsis. Res. Exp. Med. (Berl.) 191: 177-187.

Mohsin, A.K.M. and Ambak, M.A. 1983. Freshwater fishes of Peninsular Malaysia. Penerbit Universiti Pertanian Malaysia, Malaysia. 284 p.

Mophatra, B. C. and Noble, A. 1992. Liver and kidney damage in grey mullet *Liza parsia* (Hamilton and Buchanan) on exposure to an organophosphate 'Nuran'. Journal of the Marine Biology Association India 34 : 218-221.

Muller, R. and Lloyd, R. 1994. Sublethal and chronic effects of pollutants on freshwater fish. The UK inversity Press, Cambridge, England. 366 pp.

Myers, M. S., Stehr, C. M., Olson, O. P., Johnson, E., McCain, B. B., Chan, S. L., and Varanasi, U. 1994. Relationship between toxicipathic hepatic lesions and exposure to chemical contaminant in Enlish sole (*Parophry vetulus*), starry flounder (*Platichthys stellatus*), and white croaker (*Genyonemus lineatus*) from selected marine sites on the Pacific Coast, USA. Environmental Health Perspectives 102: 200-215.

Neskovic, N. K., Poleksic, V., Elezovic, I., Karan, V., and Budimir, M. 1996. Biochemical and histopathological effects of glyphosate on carp, *Cyprinus carpio* L. Bull. Environ . Contam. Toxicol. 56: 295-302.

Nimmo, H. 1985. Environmental biological processes and ecotoxicology. CRC Press LLC: London.Nowak, B. 1996. Relationship between endosulfan residue level and ultrastructural changes in the liver of catfish, *Tandanus tandanus*. Arch . Environ. Contam. Toxicol. 30: 195-202.

Olivia, C., Carmen, S., and Julian, B. 2003. Effect of lead on ALA-D activity, metallothionein levels, and lipid peroxidation in blood, kidney, and liver of the toadfish *Halobatrachus didactylus*. Ecotoxicology and Environmental Safety 55: 116-125.

Pandey, A. K. 1994. Branchial and renal lesions in the estuarine mullet, *Liza parsia*, exposed to sublethal concentration of mercury. Proceedings of the National Academy of science, India 648: 281-287.

Patil, V. T., Shinde, S. V., and Kulkarni, A. B. 1992. Histopathological changes induced by monocrotophos in the liver of the fish *Boleophthalmus dussumieri*. Environ. Ecol. 10(1): 52-54.

Patwardhan, S. A. and Gaikwad, S. A. 1991. Effect of sumithion on liver, hepatopancreas and mesenteric pancreas of freshwater fish *Gambusia affinis*. Environment and Ecology 9(2): 352-355.

Pedlar, R. M. P., tashynski, M. D., Evans, R., and Klaverkamp, J. F. 2002. Toxicological effects of dietary arsenic exposure in lake whitefish (*Coregonus clupeaformis*). Aquatic Toxicology 57: 167-189.

Phillippart, J. C. and Ruwet, J. C. 1982. Ecology and distribution of tilapias. Proceedings of the International Conference on the Biology and Culture of Tilapias 1: 15-59.

Phillips, M. J., Poucell, J., Patterson, J., and Valenria, P. 1987. The liver- an atlas and text of ultrastructural pathology. New York: Raven Press.

Ptashynski, M. D., Pedlar, R. M., Evans, R. E, Baron, C. L., and Klaverkamp, J. F. 2002. Toxicological effects of dietary nickel in lake whitefish (*Coregonus clupeaformis*). Aquatic Toxicology 58: 229-247.

Rabia, S., Mahmut, S., and Figen E. 2003. Investigation of acute toxicity of fenitrothion on peppered corydoras (*Corydoras paleatus*). Chemosphere 56: 697-700.

Radhaiah, V. and Jayantha-Rao, K. 1992. Fevalerate toxicity to the liver in a freshwater teleost, *Tilapia mossambica* Pefers. Comparative Phisiology and Ecology 17(2): 48-53.

Rainyboth, W.J. 1996. Fishes of the Cambodian Mekong. FAO Species Identification Field Guide for Fishery Purposes. FAO, Rome, 265 p.

Raj, N. R., and Sathyanesan A. G. 1987. Histopathological and biochemical changes in the liver of a teleost fish, *Channa punctatus* (Bloch) induced by a mercurial fungicide. Environmental Pollution 47: 135-145.

Rajnikant, M., and Shukla, S. P. 1996. Impact of Endosulfan on Lactate Dehydrogenase from the Freshwater Catfish*Clarias batrachus*. Pesticide Biochemistry and Physiology 57: 220-234.

Ramalingam, K., and Murabai, P. 2002. A time course test on LC_{50} determination of chlorine toxicity and its sub-lethal effects on tissue histology of *Oreochromis mossambicus*. Aquacult 3:125-130.

Rand, G. M. and Petrocelli, S. R. 1985. Fundamental of Aquatic Toxicology. London: McGrow Hill Company.

Rangaswamy, C. P. 2002. Endosulfan induced alterations in the ionic composition of blood of *Oreochromis mossambicus* (Peters). *Polln Res*, 21(4)(2002), 443-445

Rawat, D. K., Bais, V. S. and Agrawal, N. C. 2002. A correlative study on liver glycogen and endosulfan toxicity in *Heteropneustes fossilis* (Bloch.) Journal of Environmental Biology. 23: 205-207.

Roberts, R. J. 1978. Fish Pathology. 1st ed. New York: MacMillan. 13-91 pp.

Robert, K. 2001. Hand book of pesticides Toxicology Vol. 1. Academic Press, U.S.A.

Rosemary, C., Asa, B., Thomas, E. M., Dana, B. B., Martha, E. H. and Brenda, E. 2003. Cumulative Organophosphate pesticide exposure and risk assessment among pregnant women living in an agricultural community. Environmental Health perspectives 111 – 135.

Saksena, D. N. 1999. Ichthyology. Science Publisher, Inc., Enfield, U.S.A.

Sakthivel, V. and Gaiwad, S. A. 2002. Tissue histopathology of *Gambusia affinis* under dimecron toxicity. Eco. Env. Conserv. 8: 27-31.

Sakulteangtong, S. and Baiardol, P. 2001. Moritoring of agricultural toxic substances in water and sediment samples from Pakpanang River Basin. Toxic substances news and reports 28(3): 3-19.

Sakulteangtong, S. and Baiardol, P. 1996. Organophosphorus and carbamates insecticide residues in water and sediments from Joa-Praya River and Tributaries. Toxic substances news and report 28(1): 35 -42.

Sancho, L. 2002. Characterisation of choline esterase and their tissue and subcellular distribution in mussel (*Mytilus edulis*). Marine Environmental Research. 57(3): 155-169.

Sastry, K. V. and Sharma, K. 1981. Diuzinon-indnced histophatological and hematological alterations in a freshwater teleost , *Ophiocephalus punctatus*. Ecotoxicology and Environmental Safety 5: 329-340.

Schreck, E. and Moyle, C. 1990. The spectrum of hepatotoxicity. CRC Press LLC: London.

Sehwaiger, J., Fent, K., Stecher, H., Ferling, H., and Negele, R. D. 1996. Effects of sublethal concentretions of triphenyltinacetate on rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol. 30: 327-334.

Shannon, M. B., Bruce, R. W. and John, J. S. 2002. Expression of P-glycoprotein and cytochrome P450 1A in intertidal fish (*Anoplarchus* purpurescens) exposed to environmental contaminants. Aquatic Toxicology 60: 17-32.

Sonia, G. 2001. Hepatic metabolism of foreign compound. Fresno, California.

Srivastava, A. K., and Srivastava, L. P. 2002. Health risk characterisation of pyrethroid exposure: a review. J. Ecophysio Occupl Hlth 2: 153-162.

Srivastava, N., Kaushik, N., and Gupta, P. 2002. Zinc induced changes in the liver and muscle of fish *Channa punctatus* (Bloch). J. Ecophysio Occupl Hlth 2: 197-204

Stahnke, L. L., Hill, D. B., and Allen J. I. 1991. TNF-α and IL-6 in alcoholic liver disease. In: In: Thurmam, R. G. 1998. Machanisms of hepatic oxicity. II. Alcoholic liver injury involved activation of Kupffer cells by endotoxin. Am. J. Physiol. 275(38): G605-G611.

Stanley, E. M. 2003. Toxicological chemistry and biochemistry. CRC Press LLC, London.

Syed, M. N. and Chetana, V. 1993. Bioaccumulative potential and toxicity of endosulfan insecticides to non-target animals. Comparative Biochemmistry and Physiology Part C: Phamacology 105: 347-361.

Szarek, A. K., Siwicki, A., Andrzejewska, J., and Terech-Majewska, E. 2000. Effects of herbicides Asoprim and Avans on the morphological pattern in haemopoietic organ and cell-mediated immunity in European catfish (*Silurus glanis*). Marine Environmental Research 50: 311 –320.

Szarek, J., Siwicki, A., Andrzejewska, A., Terech-Majewska, E., and Banaszkiewicz, T. 2000. Effects of the herbicide Roundup on the ultrastructural pattern of hepatocytes in carp (*Cyprinus carpio*). Marine Environmental Research 50: 263-266.

Takahashi, T., Kamimura, T., and Ichida, F. 1987. Ultrastructural findings on polymorphonuclear leukocyte infiltration and acute hepatocellular damage in alcoholic hepatitis. Liver 7: 347-358.

Taki, Y. 1974. Fishes of the Lao Mekong Basin. United States Agency for International Development Mission to Laos Agriculture Division. 232 p.

Tilak, K. S., and Yacobu, K. 2002. Toxicity and effect of fenvalerate on fish *Ctenopharyngodon idellus*. J. Ecotoxicol. Environ. Monit. 12: 9-15

Tilak, K. S., Veeraiah, K., and Ramana, K. 2001. Toxicity and effect of chloropyrifos to the freshwater fish *Labeo rohita* (Hamilton). Pollution Rearch. 20: 443-445.

Tilak, K. S., Veeraiah, K., and Yacuba, K. 2001. Studies on the histopathological change in the gill, liver and kidney of *Ctenopharyngodon idellus* (Valenciennes) exposed to technical fenvalerate and EC 20%. Pollution Rearch. 20: 387-393.

Timashova, L. V. 1981. Seasonal changes in the structure of the liver of plaice, *Pleuronectes* platessa. In: Couillard, C. M., Williums, P. J., Courtenay, S. C., and Rawn, G. P. 1999. Histopathological evaluation of atlantic tomcod (*Microgadus tomcod*) collected at estrurine sites receiving pulp and paper mill effluent. Aquatic Toxicololy 44: 263-278.

Tolga, C., and Serap E. G. 2002. Evaluation of the genotoxic potential of lambda-cyhalothrin using nuclear and nucleolar biomarkers on fish cells. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 534: 93-99.

Tomlin, C. 1994. The Pesticide Manual, 10th Ed. British Crop Protection Council. Blackwell Scientific Publications, Cambridge, Massachusetts. 1341 pp.

Thomson, W. T. 1995. Agricultural Chemicals, Book III, Miscellaneous Agricultural Chemicals. Thomson Publications, Fresno, California. 209 pp.

Thomson, W. T. 1998. Agricultural Chemicals, Book I, Insecticides. Thomson Publications, Fresno, California. 269 pp.

Vesna, P., and Vesela K. 2002. Effects of Trifluralin on Carp: Biochemical and Histological Evaluation. Ecotoxicology and Environmental Safety 43: 213-221.

Vijaya, L. G., Bharathi, C., Sandeep, B. V., and Subba, R. 2002. Toxicity of endosulfan and carbaryl to a brackish water oligochaete *Pontodrilus bermudensis*. J. Ecophysio. Occupl. Hlth. 2: 39-43.

Vinay, K., Ramzi, C. R. and Stanley, L. R. 1999. Basic pathology. London.

Ware, G. W. 2000. The Pesticide Book, 5th Ed. Thomson Publications, Fresno, California. 415 pp.

Wattanasirmkit, K. 1993. Histological studies on the effects of Triphenyltin Hydroxide pesticide to liver and kidney of Catfish (Hybrid of *Clarias gariepinus* and *C. macrocephalus*). Doctor of Philosophy Thesis, Mahidol University.

Welcomme, R.L. 1988. International introductions of inland aquatic species. FAO Fish. Tech. Pap. 294. 318 p.

Wester, P. W., and Canton, J. H. 1986. Histopathological study of *Oryzias latipes* (Medaka) after long term hexachlorocyclohexane exposure. Aquatic Toxicology 9: 21-45.

William, Y. 2000. Toxicity of pollutant water in Mississippi River Basin. J. Ecophysio. Occupl. Hlth. 6: 29-33.

Wongratana, T. 1996. Taxonomy of Nile Tilapia. In: Srijunngam, J. 1998. Subchronic effects of neem Azadirachta indica A. Juss. Seed extract on female reproductive system of tilapia Oreochromis niloticus Linn. Master' s Thesis, Department of Biology, Graduate School, Chulalongkorn University. 124 pp.

Wu, W. Z., Zhou, B. S., Xu, Y., Schramm, K. W., and Kettrup, A. 1999. Cytological and Biochemical alterations in *Carassius auratus* hepatocytes from exposure to sediment contioning dioxins and related compounds. Arch. Environ. Contum. Toxicol. 37: 358-363.

Yang, J. L. and Chen, H. C. 2003. Growth retardation and histopatology of common carp (*Cyprinus carpio*) exposed to gallium. Bull. Environ. Contam. Toxicol. 71: 240-247.

Yang, X., Jha, A., Brydson, R., and Cochrane, R. C. 2004. The effects of a nickel oxide precoat on the gas bubble structures and fish -scaling resistance in vitreous enamels. Materials Science and Engineering 366: 254-261.

Yiannis, K., Tracy L. M., Gordon, C. B., and Chris D. M. 2003. Effects of the antiandrogens, vinclozolin and cyproterone acetate on gonadal development in the Japanese medaka (*Oryzias latipes*). Aquatic Toxicology 63: 391-403.

APPENDICES

Appendix A

The chemical and apparatus

A. Chemical and apparatus for histological study

Apparatus

1. Apparatus for culture fish
    - 12 L glass jar
    - 325 L glass aquaria
    - oxygen pump
    - a dip net
2. Apparatus for measure fish
    - digital weight
    - ruler
3. Apparatus for measure physical factors and water quality
    - thermometer
    - DO meter
    - pH paper
4. Apparatus for paraffin method
    - oven 37 - 60°C
    - rotary microtome
    - microtome knife
    - slide and cover slide
    - scissors
    - vial 30 ml
    - forceps
    - vacuum
    - refrigerator
    - slide warmer
    - hotplate
    - slide box

- mold
- embedding ring
- dropper
- paintbrush
- ribbon box

5. Apparatus for frozen technique
    - frozen microtome
    - microtome knife
    - slide and cover slide
    - slide box
    - forceps
    - scissors
    - paintbrush
6. Apparatus for staining
    - petridis
    - jar
    - beaker
    - tissue paper
    - forceps
7. Other apparatus
    - pipette
    - micropipette
    - cylinder
    - flask
    - hood
    - stirrer
    - glass filter funnel

Chemical

1. Chemical for paraffin method
    - 10% neutral buffer formalin
    - 70%, 90%, 95% ethyl alcohol
    - 0.5% eosin
    - heamatoxylin
    - N-butanol
    - xylene
    - paraffin wax
    - egg albumin
    - canada balsam
2. Chemical for glycogen and lipid test
    - oil red o
    - periodic acid
    - schiff ' s chemical
    - sulfurous acid
    - glycerin jelly
    - tissue media

B. Chemical and apparatus for gas chromatograph

Apparatus

1. extraction
    - 1 L separatory funnel with TFE stopcock
    - stand and clamp
    - beaker size 10 ml to 1,000 ml
    - cylinder 50 ml, 100 ml, 250 ml and 1,000 ml
    - Erlenmeyer flask
    - dropper
    - vial glass size 2 ml, 4 ml and 10 ml
    - filter whatman no.1
    - glass filter funnel
    - 200 ml size evaporatory tube (Turbo Vep tube)
    - evaporator (Turbo Vep)
2. clean up
    - 300 mm X 25 mm chromatographic column with small glass wool plug
    - beaker
    - Erlenmeyer flask
3. analysis
    - Flame Photometric Detector (FPD)
    - HP-5 (5 % diphenyl, 95 % dimethyl polysiloxane) ID 0.32 mm, Film 0.25 μm
    - HP 6890 gas chromatography
    - autosample injector 7683 series of Hewlett peckard
4. Other apparatus
    - hood
    - syringe
    - pipette

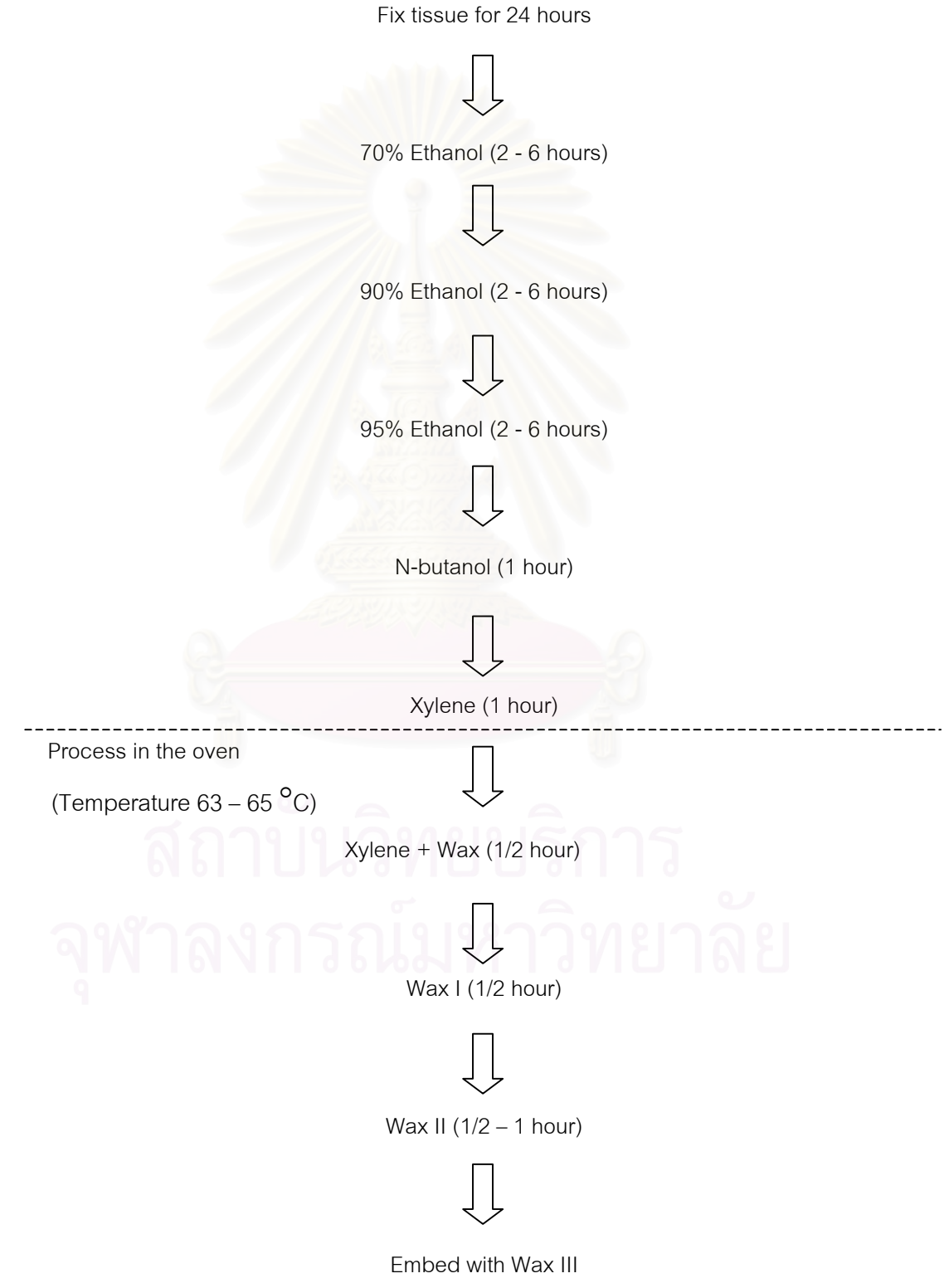
Chemical

1. extraction
    - acetone pesticides grade
    - hexane pesticides grade
    - dichloromethane pesticides grade
    - sodium sulfate
    - N_2
2. clean up
    - florisil
    - sodium sulfate
    - hexane
    - 6%, 15%, 50% and 100% petroleum ether
3. gas chromatography
    - pesticides standard
    - N_2
    - H_2
    - Air
4. Other chemical
    - distill water

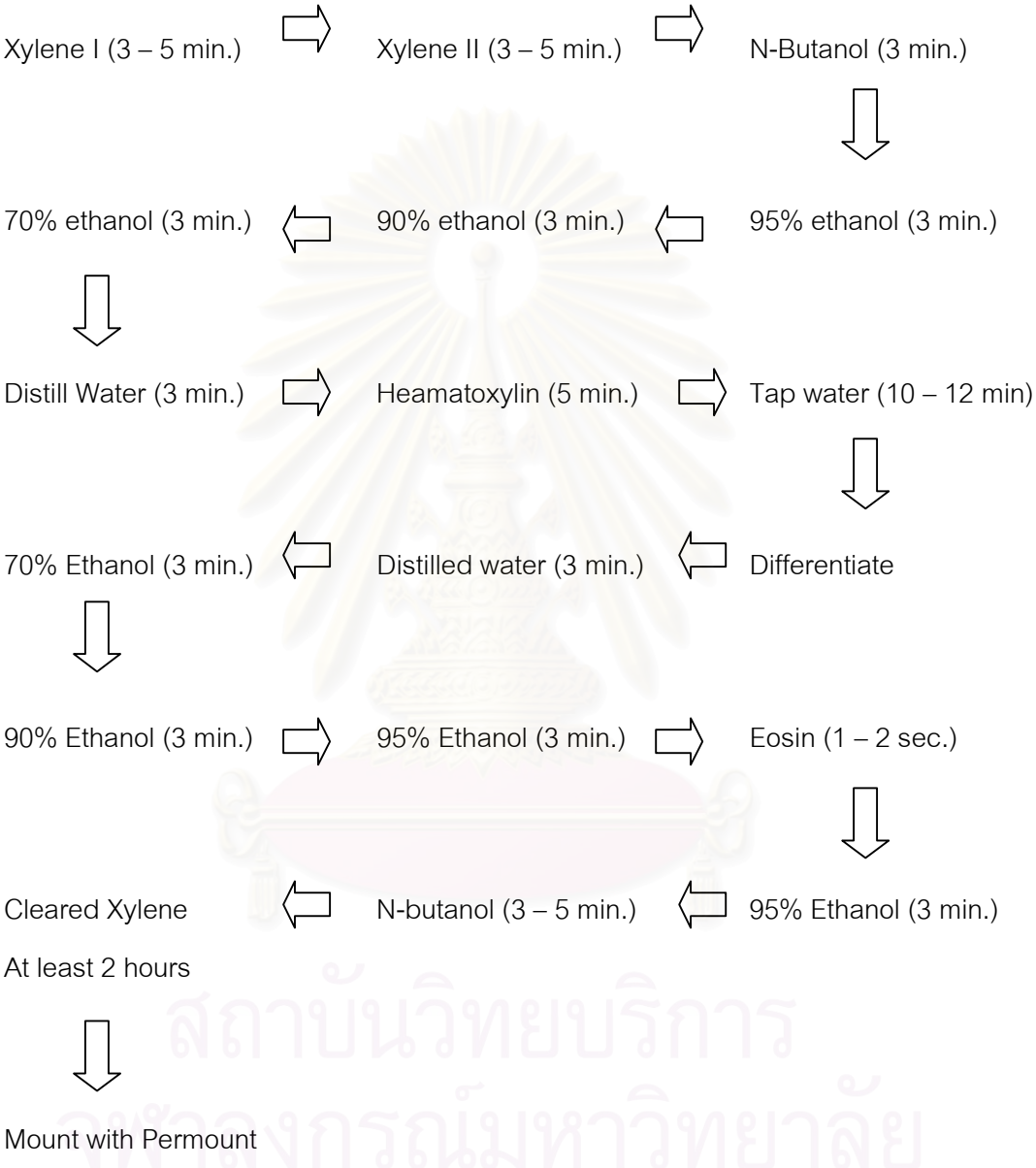
Appendix B

The process of histological study

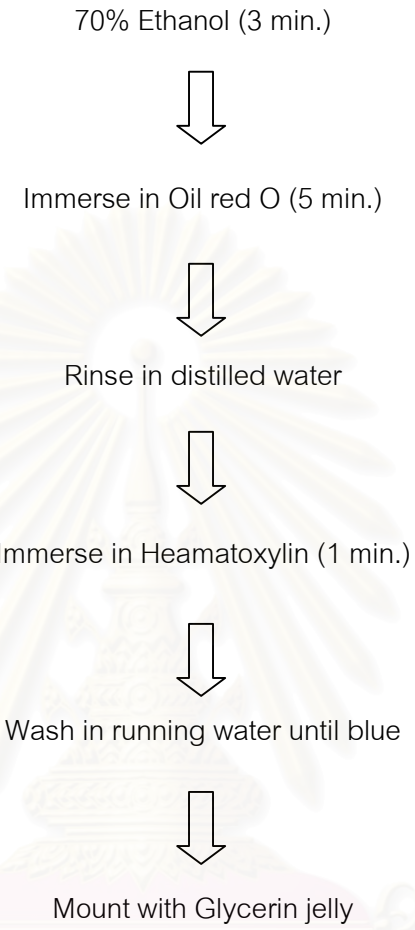
Embedding procedure



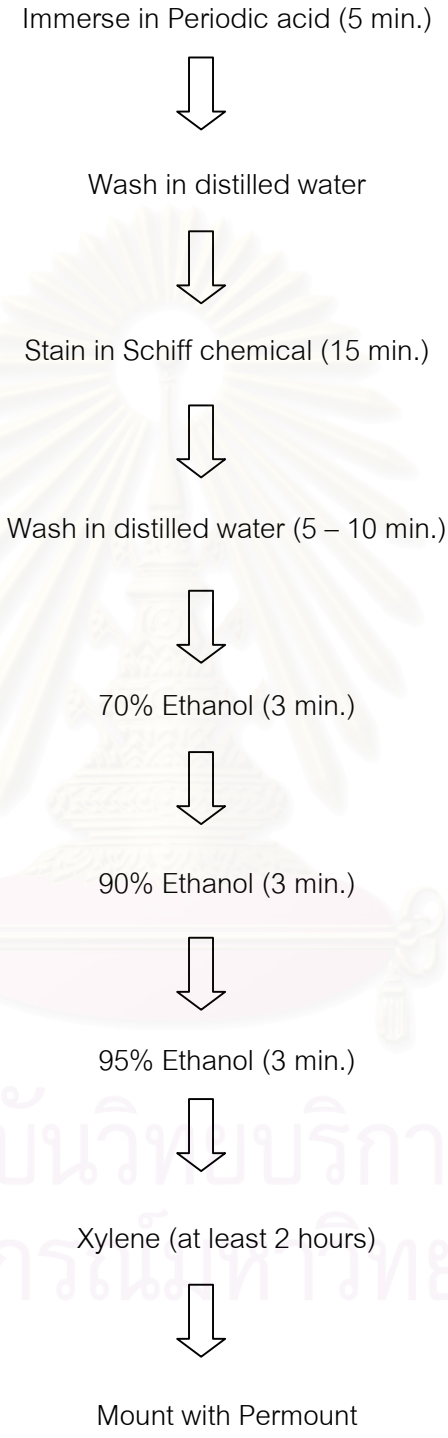
The heamatoxylin / eosin staining procedure



The Oil red O staining procedure



The PAS staining procedure



Appendix C

The data of histology

The fish data of control

	N	Minimum	Maximum	Sum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Total length	34	6.00	12.50	274.10	8.0618	.3296	1.92196
Body weight	34	2.39	19.16	261.73	7.6979	1.1139	6.49510
Liver weight	34	.030	.430	5.329	.15674	.01955	.114015
% RL	34	.00	.05	.82	.0241	.0018	.01059

The fish data of rainy season

	N	Minimum	Maximum	Sum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Total length	44	6.50	19.00	466.00	10.5909	.3981	2.64046
Body weight	44	3.34	107.05	947.76	21.5400	2.8588	18.96315
Liver weight	44	.04	1.55	7.71	.1752	.0355	.23575
% RL	44	.003	.222	.584	.01327	.00489	.032461

The fish data of dry season

	N	Minimum	Maximum	Sum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Total length	70	6.00	20.20	805.40	11.5057	.4243	3.55009
Body weight	70	4.00	98.46	1950.30	27.8614	2.8689	24.00255
Liver weight	70	.03	2.13	23.52	.3360	.0463	.38716
% RL	70	.001	.170	1.007	.01439	.00250	.020947

Mean of length, body weight and liver weight of control fish and Klong 7 fish in dry season and rainy season.

Number of fish		Length (cm) ± SE	Body weight (g) ± SE	Liver weight (g) ± SE
Control group (N=34)	N1 = 19	6.820 ± 0.150	3.500 ± 0.220	0.100 ± 0.009
	N2 = 5	7.000 ± 0.273	4.140 ± 0.467	0.060 ± 0.015
	N3 = 10	10.710 ±0.366	17.440 ± 0.509	0.290 ± 0.037
Dry season (N=70)	Site A = 18	10.120 ± 0.678	19.450 ± 4.125	0.280 ± 0.077
	Site B = 18	14.090 ± 0.246	43.210 ± 6.615	0.450 ± 0.087
	Site C = 34	10.860 ± 0.521	24.180 ± 3.678	0.300 ± 0.072
Rainy season (N=44)	Site A = 3	13.660 ± 1.855	43.770 ± 9.894	0.240 ± 0.058
	Site B = 5	11.86 ± 1.457	32.070 ± 8.648	0.210 ± 0.075
	Site C = 36	10.150 ± 0.391	18.220 ± 2.947	0.160 ± 0.042

The numbers of Klong 7 fish exhibiting specific lesions in dry season

	Site A		Site B		Site C		Total (N=32)
	Large size (N=4)	Small Size (N=6)	Large size (N=6)	Small Size (N=4)	Large size (N=6)	Small Size (N=6)	
Hyalin droplet accumulation	3	3	4	2	4	3	19
Lipid droplet accumulation	1	4	1	4	3	3	16
Foci necrosis	3	3	4	2	3	2	17
Diffuse necrosis	0	1	0	1	1	0	3
Foci and diffuse necrosis	0	0	2	0	1	1	4
Sinusoid dilate	4	4	4	3	5	3	23
Blood congestion	4	5	6	4	5	5	29
Endothelial damage	4	5	6	4	5	5	29
White blood cell Infiltration	3	3	2	3	2	2	16
Pyknotic	3	2	4	3	4	3	19
Karyolysis	3	2	3	4	5	4	21

N = The number of fish

Large size = indicate that body weight more than 40 g

Small size = indicate that body weight less than 40 g

The numbers of Klong 7 fish exhibiting specific lesions in rainy season

	Site A		Site B		Site C		Total (N=32)
	Large size (N=2)	Small Size (N=0)	Large size (N=2)	Small Size (N=1)	Large size (N=8)	Small Size (N=19)	
Hyalin droplet accumulation	1	0	1	0	2	4	8
Lipid droplet accumulation	1	0	0	0	5	4	10
Foci necrosis	0	0	0	1	2	10	13
Diffuse necrosis	0	0	0	0	0	1	1
Foci and diffuse necrosis	1	0	1	0	4	5	11
Sinusoid dilate	1	0	0	1	0	6	8
Blood congestion	1	0	1	1	5	12	20
Endothelial damage	1	0	1	1	8	16	27
White blood cell infiltration	1	0	1	0	3	13	17
Pyknotic	1	0	0	1	3	11	16
Karyolysis	1	0	1	1	7	11	21

N = The number of fish

Large size = indicate that body weight more than 40 g

Small size = indicate that body weight less than 40 g.

The degree of lesion of Klong 7 fish liver alteration between site studies in dry season season

	Control	Site A	Site B	Site C
Hyalin droplet accumulation	-	++	++	++
Lipid droplet accumulation	++	++	++	++
Foci necrosis	-	++	++	++
Diffuse necrosis	-	+	+	+
Foci and diffuse necrosis	-	-	+	+
Sinusoid dilation	-	++++	+++	+++
Blood congestion	-	++++	++++	++++
Endothelial damage	-	++++	++++	++++
White blood cell infiltration	-	++	++	++
Pyknotic	-	++	+++	++
Karyolysis	-	++	+++	+++

-	Not present
+	Mild degree of lesion
++	Moderately degree of lesion
+++	Strongly degree of lesion
++++	Extremely degree of lesion

The degree of lesion of Klong 7 fish liver alteration between site studies in rainy season

	Control	Site A	Site B	Site C
Hyalin droplet accumulation	-	++	++	++
Lipid droplet accumulation	++	++	-	++
Foci necrosis	-	-	++	++
Diffuse necrosis	-	-	-	+
Foci and diffuse necrosis	-	++	++	++
Sinusoid dilate	-	++	++	+
Blood congestion	-	++	+++	++
Endothelial damage	-	++	+++	++++
White blood cell infiltration	-	++	++	++
Pyknotic	-	++	++	++
Karyolysis	-	++	+++	+++

-	Not present
+	Mild degree of lesion
++	Moderately degree of lesion
+++	Strongly degree of lesion
++++	Extremely degree of lesion

Comparison violent of liver alteration between body sizes of Klong 7 fish

	Control (N=15)	Dry season season		Rainy season	
		Large size (N=16)	Small size (N=16)	Large size (N=12)	Small size (N=20)
Hyalin droplet accumulation	-	+++	++	++	+
Lipid droplet accumulation	++	+	+	+	+
Foci necrosis	-	++	++	+	++
Diffuse necrosis	-	+	+	-	+
Foci and diffuse necrosis	-	+	+	++	+
Sinusoid dilate	-	++++	++	+	++
Blood congestion	-	++++	++++	++	++
Endothelial damage	-	++++	++++	+++	+++
White blood cell infiltration	-	++	++	++	++
Pyknotic	-	+++	++	++	++
Karyolysis	-	+++	+++	+++	+++

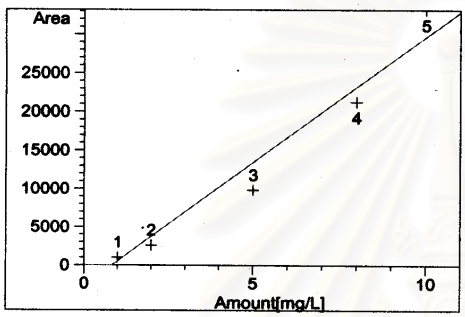
-	Not present l
+	Mild degree of lesion
++	Moderately degree of lesion
+++	Strongly degree of lesion l
++++	Extremely degree of lesion

Appendix D

The data of Gas chromatograph

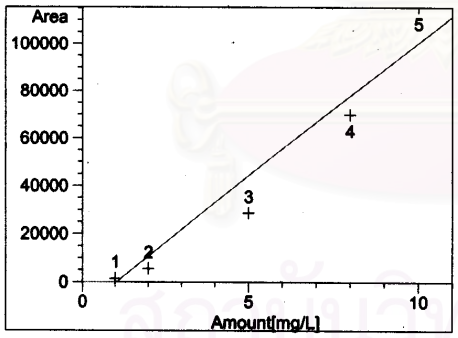
Calibration curve of organophosphate insecticides

Thionazin



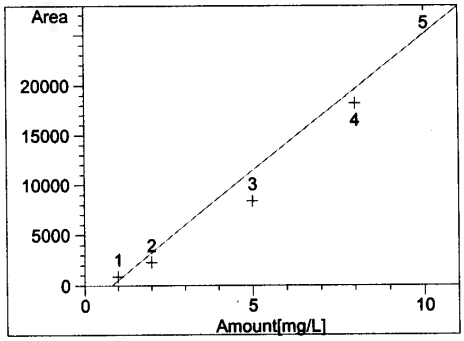

```
Thionazin at exp. RT: 7.123
FPD1 B,
Correlation:              0.97806
Residual Std. Dev.: 3110.94573
Formula: y = mx + b
      m:   3239.20556
      b:  -2743.36856
      x: Amount [mg/L]
      y: Area
```

Dimethoate



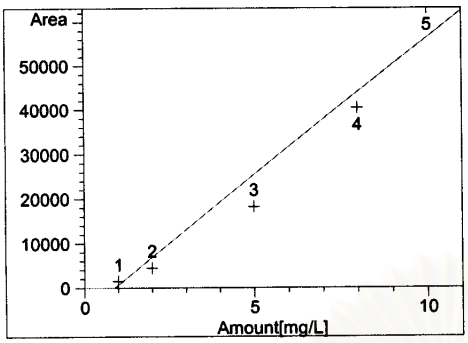

```
Dimethoate at exp. RT: 8.396
FPD1 B,
Correlation:              0.96715
Residual Std. Dev.: 13284.31466
Formula: y = mx + b
      m:  11208.72212
      b: -11775.86144
      x: Amount [mg/L]
      y: Area
```

Ethylparathion



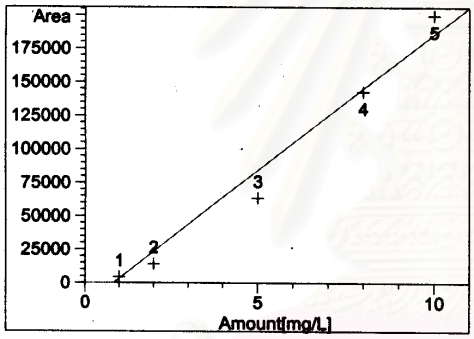

```
Ethylparathion at exp. RT: 11.041
FPD1 B,
Correlation:              0.97982
Residual Std. Dev.: 2514.81450
Formula: y = mx + b
      m:   2734.18900
      b:  -2232.76283
      x: Amount [mg/L]
      y: Area
```

Famphur



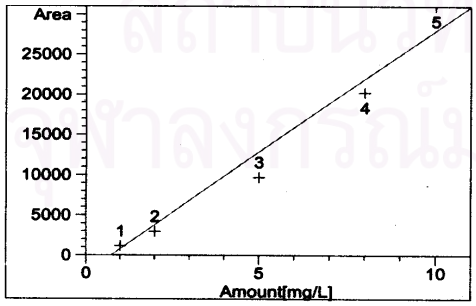
Famphur at exp. RT: 13.938
FPD1 B,
Correlation: 0.97704
Residual Std. Dev.: 6080.61621
Formula: y = mx + b
m: 6184.27527
b: -5523.36696
x: Amount [mg/L]
y: Area

Disulfoton



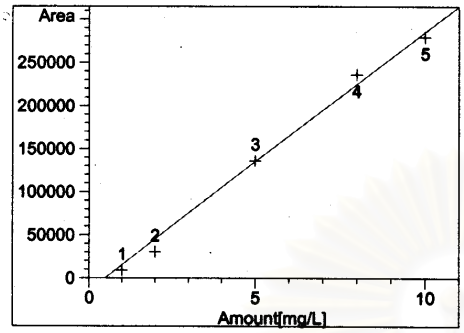
Disulfoton at exp. RT: 9.245
FPD1 B,
Correlation: 0.98511
Residual Std. Dev.: 15970.95508
Formula: y = mx + b
m: 20290.97838
b: -17212.82939
x: Amount [mg/L]
y: Area

o, o, o- Triethylphosphothioate



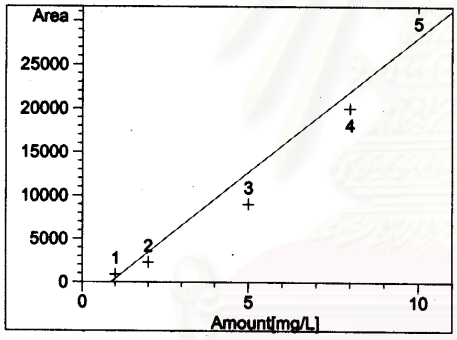
o,o,o Triethylphosphothioate at exp. RT: 2.018
FPD1 B,
Correlation: 0.98149
Residual Std. Dev.: 2659.66743
Formula: y = mx + b
m: 3023.03952
b: -2257.04661
x: Amount [mg/L]
y: Area

Sulfotepp



Sulfotepp at exp. RT: 8.045
FPD1 B,
Correlation: 0.99573
Residual Std. Dev.: 12544.92611
Formula: y = mx + b
m: 30002.49279
b: -14824.65641
x: Amount [mg/L]
y: Area

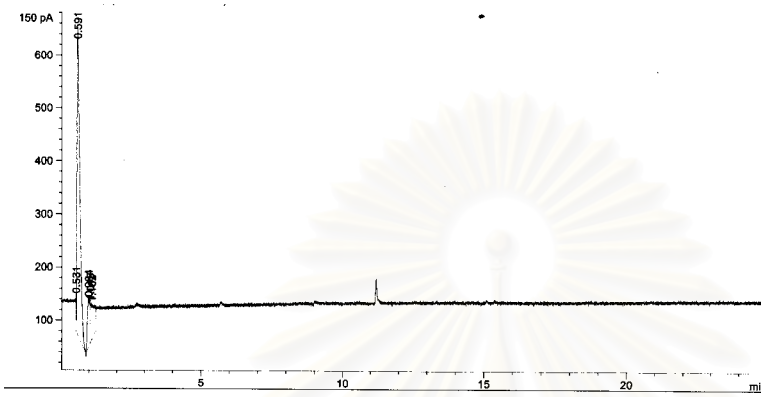
Methylparathion



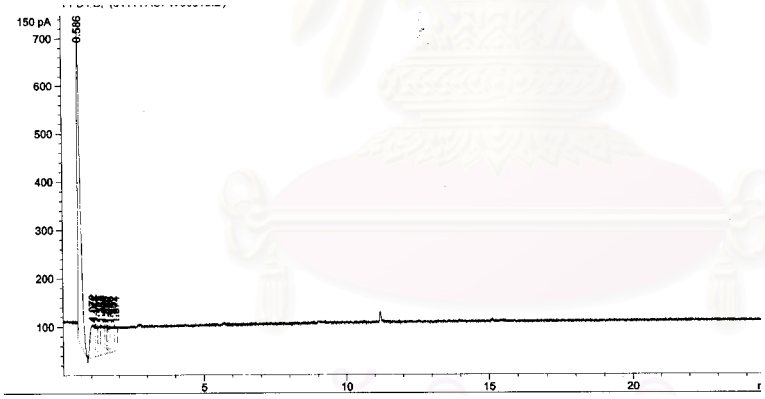
Methylparathion at exp. RT: 10.064
FPD1 B,
Correlation: 0.97597
Residual Std. Dev.: 3097.59646
Formula: y = mx + b
m: 3076.84550
b: -2702.56953
x: Amount [mg/L]
y: Area

Chromatogram of organophosphate insecticides residual at klong 7 from March to December 2004.

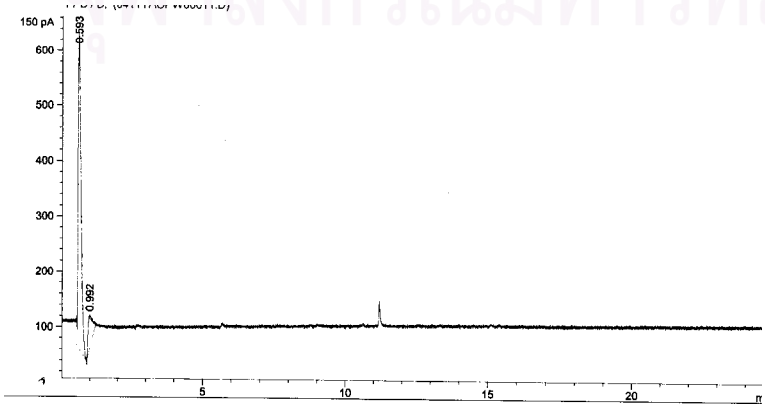
March



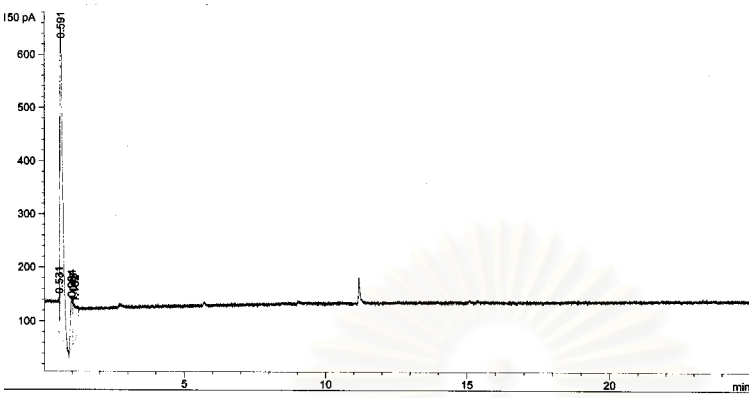
April



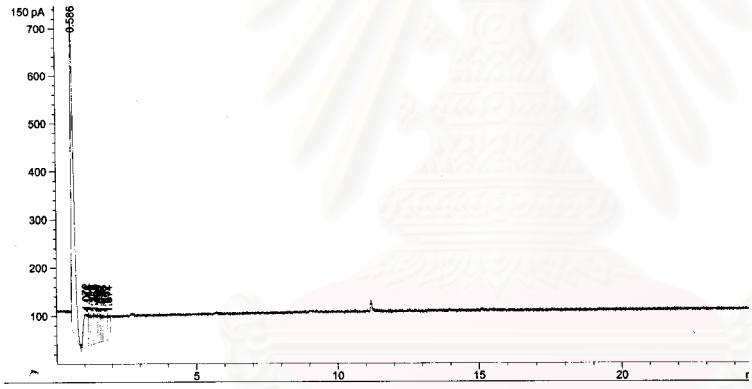
May



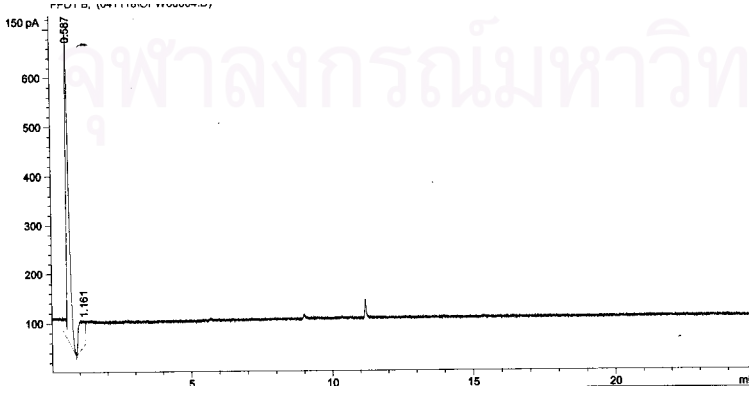
June



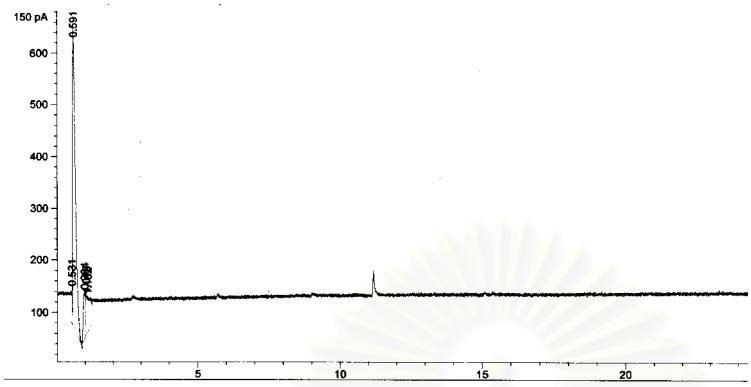
July



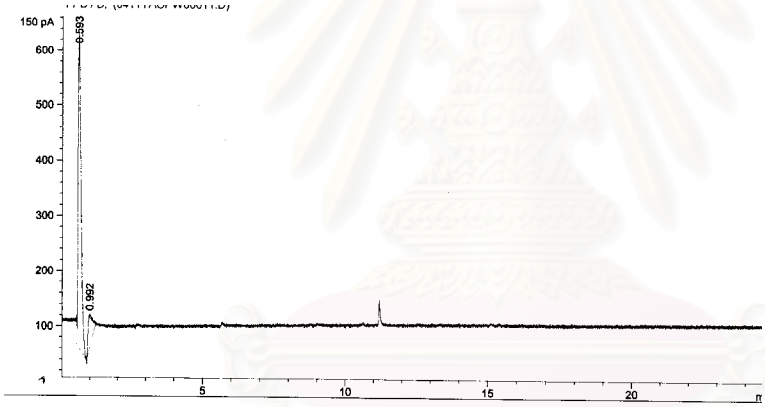
August



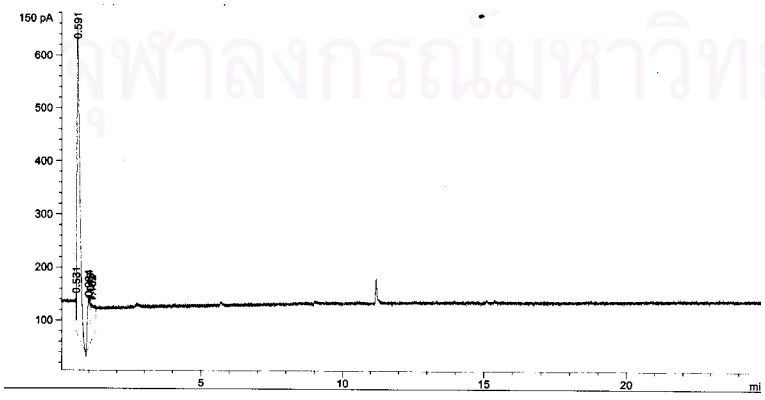
September



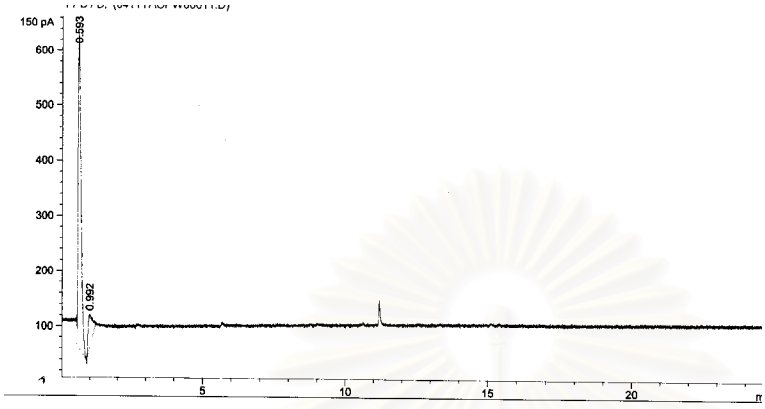
October



November



December



VITAE

Miss Watiporn Yenchum was born on November 21, 1979, in Nakhon Nayok Province. She received a Bacherlor's degree of Science in Biology, from Chulalongkorn University, in 2001. She continued her study for M.Sc. in zoology, Department of Biology, Faculty of Science, Chulalongkorn University in 2002 and completed program in 2004.