สารกำจัดแมลงตกค้างกลุ่มออร์กาโนคลอรีนในระบบนิเวศแหล่งน้ำ และการประเมินความเสี่ยงด้านสุขภาพของชุมชนเกษตรกรรมท้องถิ่น

นายวัฒน์สิทธิ์ ศิริวงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ORGANOCHLORINE PESTICIDE RESIDUES IN AQUATIC ECOSYSTEM AND HEALTH RISK ASSESSMENT OF LOCAL AGRICULTURAL COMMUNITY

Mr. Wattasit Siriwong

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Environmental Management
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Thesis Title	ORGANOCHLORINE PESTICIDE RESIDUES IN AQUATIC ECOSYSTEM AND HEALTH RISK ASSESSMENT OF LOCAL AGRICULTURAL COMMUNITY
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วัฒน์สิทธิ์ ศิริวงศ์: สารกำจัดแมลงตกค้างกลุ่มออร์กาโนคลอรีนในระบบนิเวศแหล่งน้ำ และการประเมินความเสี่ยงด้านสุขภาพของชุมชนเกษตรกรรมท้องถิ่น (ORGANOCHLORINE PESTICIDE RESIDUES IN AQUATIC ECOSYSTEM AND HEALTH RISK ASSESSMENT OF LOCAL AGRICULTURAL COMMUNITY) อ. ที่ปรึกษา: ผศ. ดร. กำธร ธีรคุปต์, อ. ที่ปรึกษาร่วม: ศ. ดร. มาร์ค รอบสัน, อ. ดร. ดวงแข สิทธิเจริญชัย, 148 หน้า

การศึกษาสารกำจัดแมลงตกค้างกลุ่มออร์กาโนคลอรีน ได้แก่ กลุ่มเอชซีเอช กลุ่มเฮปตะคลอร์และเฮปตะคลอร์อีป็อกไซด์ กลุ่มดีดีทีและอนุพันธ์ กลุ่มอัลดรินและดิลดริน กลุ่มเอ็นโดซัลแฟน กลุ่มเอ็นดรินและเอ็นดรินอัลดีไฮด์ และ เมทท็อกซี่คลอร์ ในระบบนิเวศแหล่งน้ำ บริเวณพื้นที่เกษตรกรรมรังสิต คลองเจ็ด จังหวัดปทุมธานี ได้ดำเนินการเก็บตัวอย่างตั้งแต่เดือนมิถุนายน พ.ศ. 2547 ถึง เดือนกุมภาพันธ์ 2550 ตัวอย่างที่ทำการศึกษาประกอบด้วย ตะกอนดิน น้ำ แพลงตอน (แพลงตอนพืชและสัตว์) พืชน้ำ กุ้ง หอย และ ปลา ซึ่งทำการสกัดสารตกค้างในตัวอย่างแต่ละชนิดด้วยวิธีการสกัดแบบรวม (multiresidues extraction) และวิเคราะห์ปริมาณสารตกค้างด้วยเครื่องแกสโครมาโทรกราฟี ชนิดหัวตรวจแบบไมโครอิเล็คตรอนแคปเจอร์ พบว่ามีปริมาณสารกลุ่มออร์กาโนคลอรีนตกค้างในระบบนิเวศแหล่งน้ำคลองเจ็ดในระดับหนึ่งในพันล้านส่วน (พีพีบี) เมื่อเปรียบเทียบปริมาณออร์กาโนคลอรีนตกค้างเฉลี่ย พบว่าปริมาณเอนโดซัลแฟนรวมในน้ำ พืชน้ำ (8 ชนิด) และปลา (41 ชนิด) มีค่าสูงกว่าสารชนิดอื่น สำหรับตะกอนดิน แพลงตอน และสัตว์ไม่มีกระดูกสันหลัง (3 ชนิด) พบว่ามีปริมาณดีดีทีและอนุพันธ์ตกค้างเฉลี่ยสูงสุด นอกจากนี้พบว่าปริมาณการตกค้างของสารกลุ่มออร์กาโนคลอรีนส่วนใหญ่ในสิ่งมีชีวิตมีรูปแบบดังนี้ แพลงตอน < พืชน้ำ < สัตว์มีกระดูกสันหลัง (ปลา) < สัตว์ไม่มีกระดูกสันหลัง (กุ้ง และ หอย) ตามลำดับ และมีรูปแบบในสิ่งแวดล้อมทางกายภาพคือ น้ำ < ตะกอนดิน แม้ว่าสารกำจัดแมลงกลุ่มออร์กาโนคลอรีนได้ถูกยกเลิกการใช้ทั้งหมดในประเทศไทยแล้ว ยังพบว่ามีการสะสมและถ่ายทอดในแต่ละลำดับขั้นของการบริโภค (trophic level) เพิ่มขึ้นผ่านสายใยอาหารของระบบนิเวศแหล่งน้ำ ด้วยตระหนักถึงผลกระทบต่อสุขภาพมนุษย์ งานวิจัยนี้จึงทำการประเมินความเสี่ยงสูงสุดต่อสุขภาพของประชากรในพื้นที่ชุมชนคลองเจ็ดซึ่งได้รับสารพิษตกค้างกลุ่มออร์กาโนคลอรีนจากการบริโภคสิ่งมีชีวิตในคลองเจ็ดเท่านั้น พบว่า การบริโภคปลาบางชนิดของกลุ่มประชากรในคลองเจ็ด มีความเสี่ยงสัมพันธ์ต่อการเกิดมะเร็ง ซึ่งสาเหตุจากการปนเปื้อนด้วยสารอัลฟ่าเอชซีเอช เบต้าเอชซีเอช เฮปตะคลอร์ เฮปตะคลอร์อีป็อกไซด์ อัลดริน ดริลดริน ดีดีอี ดีดีดี และดีดีที เช่นเดียวกับการบริโภคกุ้งฝอย หอยขม ผักบุ้ง ผักกระเฉด และสายบัว มีความเสี่ยงสัมพันธ์ต่อการเกิดมะเร็ง ซึ่งมีสาเหตุจากการปนเปื้อนด้วยสารอัลฟ่าเอชซีเอช เบต้าเอชซีเอช เฮปตะคลอร์ เฮปตะคลอร์อีป็อกไซด์ อัลดริน และดริลดริน ดังนั้น ภาครัฐและประชาชนในพื้นที่เกษตรกรรมรังสิต ควรมีแนวทางการจัดการอย่างเหมาะสมเพื่อลดความเสี่ยงที่อาจจะเกิดจากสารตกค้างกลุ่มออร์กาโนคลอรีนต่อไป

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต .....

ปีการศึกษา 2549 ลายมือชื่ออาจารย์ที่ปรึกษา .....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

4789708220 : MAJOR ENVIRONMENTAL MANAGEMENT
KEY WORD : ORGANOCHLORINE PESTICIDE/ AQUATIC ECOSYSTEM/ FOOD WEB/ BIOCONCENTRATION/ BIOACCUMULATOION/ BIOMAGNIFICATION/ HEALTH RISK ASSESSMENT/ RANGSIT AGRICULTURAL AREA

WATTASIT SIRIWONG : ORGANOCHLORINE PESTICIDE RESIDUES IN AQUATIC ECOSYSTEM AND HEALTH RISK ASSESSMENT OF LOCAL AGRICULTURAL COMMUNITY. THESIS ADVISOR: ASST. PROF. KUMTHORN THIRAKHUPT, PH.D., THESIS COADVISOR: PROF. MARK G. ROBSON, PH.D., DUANGKHAE SITTHICHAROENCHAI, PH.D., 148 pp.

The study of organochlorine pesticide residues (OCPRs) such as HCHs, heptachlor and heptachlor epoxide, DDT and derivatives, total endosulfan, endrin and endrin aldehyde, and methoxychlor in aquatic ecosystem was conducted from June 2004 to February 2007 at Khlong 7 (canal), Rangsit aricutural area, Pathum-Thani Province. The OCPRs in various samples (sediment, water, plankton (phyto- and zoo-plankton), aquatic plant, shrimp, freshwater snail, and fish) were extracted using multiresidue extraction method and then analyzed by gas chromatography with micro electron capture (μ-ECD) detector. The results showed that low concentrations of OCPRs in Khlong 7 aquatic ecosystem were found, in part per billion (ppb) levels. The average concentration of OCPRs compared in various matrices indicated that total endosulfan was the highest in water, aquatic plants (8 species), and fish (41 species). On the other hand, DDT and derivatives was the highest detected in plankton, and invertebrates (3 species). In particular, the distribution pattern of OCPRs in aquatic organisms was planktons < aquatic plants < vertebrates (fish) < invertebrates (shrimp and freshwater snail), respectively. Generally, OCPRs distribution pattern in the physical environment was water < sediment. Even though organochlorine pesticides were banned in Thailand, the accumulation and transformation existed in the aquatic food web from the lowest up to the highest trophic level. Concerning human health, this study thus assessed the human health risk of OCPRs associated with aquatic organisms consumption from Khlong 7, based on a plausible worst-case scenario. The results showed that some fish consumption of local population at Khlong 7 could be related to a cancer risk causing by α-, β-HCH, heptachlor, heptachlor epoxide, aldrin, dieldrin, DDD, DDE, and DDT. Likewise, the consumptions of Lanchester's freshwater prawn *Macrobrachium lanchesteri*, freshwater snail *Filopaludina mertensi*, swamp morning-glory *Ipomomea aquatica*, neptunia *Neptunia oleracea*, and water lily *Nymphaea lotus* were at risk from α-, β-HCH, heptachlor, heptachlor epoxide, aldrin, and dieldrin. Therefore, the authorities and local communities should have the appropriate strategies for the reduction of health risk.

Field of study Environmental Management Student's signature Wattasit Siriwong.
Academic year 2006 Advisor's signature K. Thirakhupt
Co-advisor's signature
Co-advisor's signature Duangkhae Sitthicharoenchai

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LIST OF ABBREVIATIONS

AOAC	Association of Analytical Communities
AR grade	Analytical Reagent grade
ASE	Accelerated Solvent Extractor
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BMF	Biomagnification Factor
CSF	Cancer Flope Factor
DDD	1, 1 - dichloro- 2, 2 - bis (p - chlorophenyl) ethane
DDE	1, 1 - dichloro- 2, 2 - bis (p - chlorophenyl) ethylene
DDT	1, 1, 1 - trichloro- 2, 2 - bis (p - chlorophenyl) ethane
ECD	Electron Capture Detector
g	Gram
GC	Gas Chromatography
HCH	hexachlorocyclohexanes
IRIS	Integrated Risk Information System
L	Liter
LLE	Liquid - liquid Extraction
LOAEL	Lowest Observed Adverse Effect Limit
LOD	Limit of Detection
LOQ	Limit of Quantitation
MDL	Method Detection Limit
mg	Milligram
mL	Milliliter
MRL	Maximum Residue Limit
ng	Nanogram
NOEL	No Observable Effects Limit
OCP	Organochlorine Pesticide
OCPR	Organochlorine Pesticide Residue
ppb	part per billion
ppm	part per million
PR grade	Pesticide Reagent grade
Rfd	Referent dose

RSD	Relative Standard Deviation
SOP	Standard Operating Procedure
SPE	Solid Phase Extraction
USEPA	United States Environmental Protection Agency
wt.	weight
μ	micro
α	alpha
β	beta
δ	delta
ε	epsilon
γ	gamma

CHAPTER I

INTRODUCTION

1.1 Theoretical Background

Over the past few decades, Thailand's agricultural sector has shifted from labor- to machine- intensive farming practices. The heavy use of pesticides to protect crops has been essential in sustaining high crop yields. Since 1957, the importation of chemical substances such as organochlorines, organophosphates and carbamates has increased rapidly. Imported pesticides rose from approximately 2,000 tons of active ingredients in 1957 to approximately 4,000 tons in 1962 and then 37,039 tons in 2001 (NIP/POPs Coordination, 2005). As a result, many hazardous effects to human health and the environment have been reported (Thirakhupt *et al.*, 2006; Matsumura *et al.*, 1992). This is particularly true for organochlorine pesticides (OCPs), which can cause severe damage to living and non-target living organisms in the environment. OCPs are able to resist biodegradation and can be transferred through food chains by two basic routes; the transport of dissolved contaminants across biological membrane channels and the ingestion of contaminated food or sediment particles that are transported across the gut. For upper-trophic-level species, the ingestion of contaminated prey is the predominant route of exposure, which has rarely reported in Thailand. For this reason, it is necessary to investigate the present situation to determine risk to human health and the ecosystem.

Topographically, the Rangsit agricultural area is located in central Thailand in the Pathum Thani Province. It has an irrigation-network-system, consisting of 14 sub-canals (Khlong) which are divided into the upper and the lower part by Rangsit-Prayulasakdi canal. The study area is situated at Khlong 7, a 20-km man-made sub-canal, on the upper part of the irrigation-network-system. It links Raphi Phat canal at the upstream side and Rangsit-Prayulasakdi canal at the downstream side (Figure 1.1). The Rangsit irrigation-network-system supports agricultural activities such as paddy fields, which is approximately 70 percent of the total province's land use (Office of Agricultural Economics, 2002), vegetable farms, fruit orchards, and fisheries. Various pesticides have been applied to this area over the past fifty years, particularly organochlorine pesticides. Due to low cost and versatility in controlling various pests,

organochlorine pesticides are still used for agricultural, medical, and urban pests despite the bans and usage restrictions placed on organochlorine pesticides from the 1970s through the 1980s. The concentration and composition of pesticide residues varies daily in the canal water, ranging from undetectable to ppb levels. The variation in detection levels may be due to pesticide drainage from rice fields, microbial decomposition in water, their adsorption onto suspended matter, etc. Thus, bioaccumulation and biomagnification patterns of pesticide residues in canal ecosystems are complicated and can lead to the disturbance of canal ecosystems.

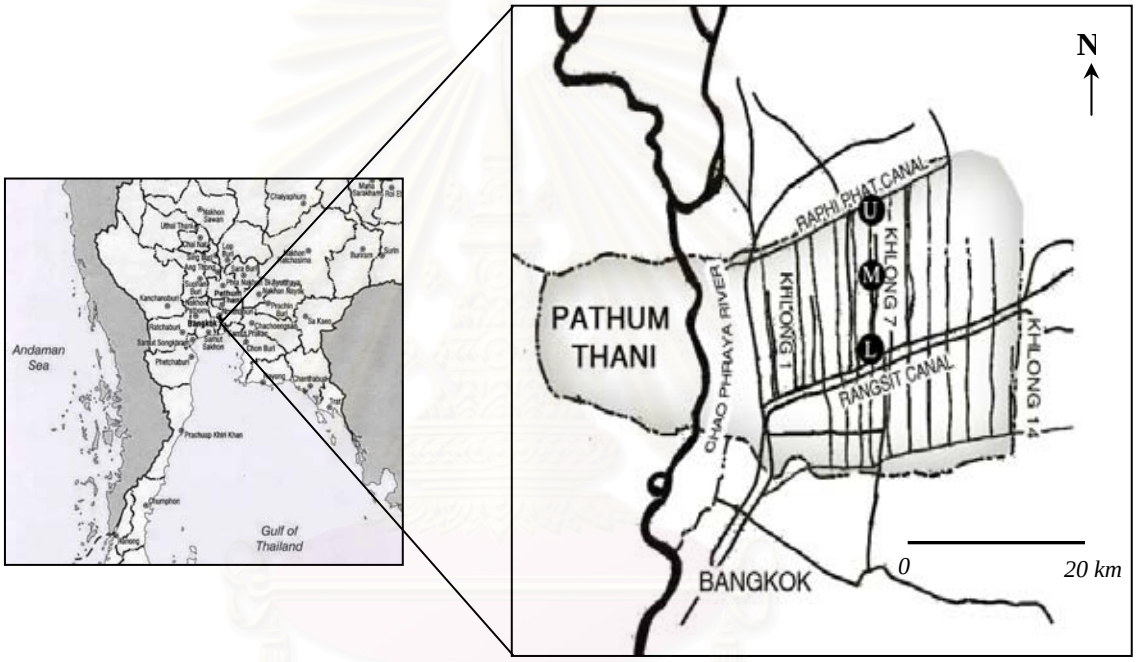


Figure 1.1 Map of Rangsit agricultural area, Pathum Thani Province.
The study area is at khlong 7 where (U) = the upper stream,
(M) = middle stream, and (L) = lower stream

1.2 Objectives

1.2.1 To examine the contamination of Organochlorine Pesticide Residues (OCPRs) in water, sediment, and dominant aquatic organisms in Khlong 7.

1.2.2 To investigate the biomagnification pathways of OCPRs in aquatic food webs at Rangsit Khlong 7.

1.2.3 To evaluate the human health risks of the Khlong 7 local community.

1.2.4 To set up the management recommendations for Khlong 7 local community.

1.3 Hypothesis

1.3.1 OCPRs still exist in various matrices of Khlong 7 ecosystem although most organochlorine pesticides have been banned.

1.3.2 OCPRs through the aquatic food web of Khlong 7 are significantly biomagnified.

1.3.3 Some local people at Khlong 7 are at risk of OCPRs contamination.

1.4 Scope of Study

1.4.1 Water, sediment, and aquatic organisms had been collected at Khlong 7, Rangsit agricultural area, Pathum Thani province from 3 sites located at the upper stream (U), middle stream (M), and lower stream (L) from May 2004 to February 2007.

1.4.2 The extraction of 17 kinds of OCPRs; α-, γ-, β- and δ-HCH, heptachlor, heptachlor epoxide, aldrin, α-endosulfan, γ-endosulfan, endosulfan sulfate, 4,4'-DDE, 4,4'-DDD, 4,4'-DDT, dieldrin, endrin, endrin aldehyde, and methoxychlor were performed in all matrices.

1.4.3 The biomagnification of OCPRs through the food web were investigated.

1.4.4 The human health risks from aquatic organism consumptions were evaluated and appropriate management strategies were considered.

CHAPTER II

LITERATURE REVIEWS

2.1 Organochlorine Pesticides

Organochlorine pesticide (OCP) is a large group of synthetic chemicals with considerable diversity of structure, property, and usage. They are stable organic compounds of very low water solubility and high lipophilicity. Some of them are highly persistent in their original forms or as stable metabolites and are considered acting as environmental hormones, which disrupt the reproductive cycle of humans and wildlife (Colborn and Smolen, 1996).

Most OCPs are classified as Persistent Organic Pollutants (POPs) on Stockholm Convention: DDT, Aldrin, Dieldrin, Endrin, Chlordane, Heptachlor, Hexachlorobenzene, Mirex and Toxaphene. The use of these compounds for agriculture, public health, and other purposes had been banned because of the hazards to human health and to the environment. However, some of these chemicals are still frequently or on a limited scale in some developing countries. (Thirakhupt *et al*., 2006; NIP/POPs Coordination, 2005)

2.1.1 Organochlorine Pesticide Groups

Organochlorine pesticides have been identified by carbon ordering of their molecular structures and have been divided into three groups (Thirakhupt *et al*., 2006a):

(1) Diphenyl Aliphatic Group

Diphenyl aliphatics include compounds such as dichloro-diphenyl-trichloroethane (DDT) and its related compounds methoxychlor and dicofol. The insecticidal properties of DDT were discovered by Paul Muller of Ciba-Geigy in 1939. DDT is one of the more well known insecticides and was mainly used for vector control during World War II. It came to be widely used thereafter for the control of agricultural pests, vectors of disease (e.g. malarial mosquitoes), and ectoparasites of farm animals including industrial and household insect pests. Because of its low water solubility, DDT has been formulated as an emulsifiable

concentrate for application as a spray. DDT has an acute oral LD_{50} of 250 mg/kg for rats and is considered to be moderately toxic to vertebrates. It has been shown to cause eggshell thinning in some sensitive species of birds at very low doses and has estrogenic effects that can cause endocrine disruption in animals. Kelthane (dicofol) is an example of a pesticide related in structure to DDT which has been marketed as an acaricide. Kelthane has weak insecticidal activity with limited persistence, but there is evidence that it may also act as an endocrine disruptor in vertebrates.

(2) Chlorinated Cyclodienes Group

Chlorinated cyclodiene insecticides were introduced during the 1950s. Some of them have both high toxicity to vertebrates and marked biological persistence thus, giving rise to some serious environmental problems. Chlorinated cyclodienes are synthesized by the Diels-Alder reaction. Aldrin, dieldrin, and heptachlor are examples of cyclodiene insecticides with acute oral LD_{50} for rats of about 40-60 mg/kg. Chlordane is a similar chemical, but is of lower vertebrate toxicity. Endrin and, to a lesser extent, endosulfan are of very high vertebrate toxicity, but limited biological persistence. In general, the cyclodienes resemble DDT in being stable lipophilic solids of very low water solubility, but differ from it in their mode of action. Endosulfan is an exception to this rule, having appreciable water solubility.

Cyclodienes were introduced into the western countries during the 1950s and were used in diverse formulations for many different purposes. Because of their water insolubility, emulsifiable concentrates and wettable powders were the formulations normally used for spraying. Sprays were used to control certain crop pests and to control vectors to prevent spread of diseases. Cyclodienes were also used in dips and sprays to control ectoparasites of livestock and were widely used as seed dressings for cereals and other crops. The use of aldrin, dieldrin, and heptachlor for the latter purpose has caused very serious ecological consequences through food chains and food webs, including contamination in soil, water, and groundwater.

(3) Hexachlorocyclohexanes (HCH) Group

HCH has similar properties to other organochlorine insecticides, but it is 100 times more polar and water soluble than DDT. HCH is classified into alpha, beta, gamma, and delta isomers. Emulsifiable concentrates of HCH have been used for

controlling agricultural pests and parasites on farm animals. It has also been used as an insecticidal seed dressing. HCH is moderately toxic to rats (LD_{50} 60-250 mg/kg).

2.2.2 Organochlorine Pesticides Properties (Thirakhupt *et al*., 2006a)

(1) Aldrin and Dieldrin

Aldrin and dieldrin are the common names of two structurally similar compounds that were once used as insecticides. They are chemicals made in the laboratory and do not occur naturally in the environment.

The scientific name for aldrin is 1,2,3,4,10,10-hexachloro-1,4,4α,5,8,8α-hexahydro-1,4-endo,exo-5,8-dimethanonaphthalene. Technical-grade aldrin is composed of not less than 85.5% aldrin. The trade names used for aldrin include Aldrec, Aldrex, Drinox, Octalene, Seedrin, and Compound 118. The scientific name for dieldrin is 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-1,4-endo,exo-5,8 dimethanonaphthalene. Technical-grade dieldrin is composed of not less than 85% dieldrin. The trade names used for dieldrin include Alvit, Dieldrix, Octalox, Quintox, and Red Shield.

Pure aldrin and dieldrin are white powders while technical-grade aldrin and dieldrin are tan powders. Aldrin and dieldrin slowly evaporate in the air with aldrin evaporating more readily than dieldrin. Both aldrin and dieldrin have mild chemical odors. Residues have been found in soil, water, and buildings where these compounds were used to kill termites. These compounds have also have been found in plants and animals near hazardous waste sites. In the past, aldrin and dieldrin were disseminated into the environment when farmers used these compounds to kill crop pests and when exterminators used them to kill termites, resulting in aldrin and dieldrin still being present in the environment. Sunlight, other physical factors, and microorganisms in the environment can convert aldrin to be dieldrin. Therefore, dieldrin can be found in places where aldrin was originally released. The half-life of dieldrin in temperate soil is approximately 5 years. Most dieldrin in the environment attaches to soil and to sediment at the bottom of lakes, ponds, and streams and may exist attached to soil unchanged for many years. Water does not easily remove dieldrin from soil and dieldrin does not dissolve easily in water making it difficult to detect high

concentrations in water. Plants can take up dieldrin from the soil and store it in their leaves and roots. Fish and animals that consume dieldrin-contaminated materials store a large amount of the dieldrin in their fat tissue. Many carnivorous animals have higher levels of dieldren in their fat tissues than herbivorous animals. Dieldrin can migrate long distances by attaching to dust particles which can be transported by the wind. In the air, dieldrin is converted to photodieldrin within a few days.

(2) Hexachlorocyclohexane (HCH)

Hexachlorocyclohexane also known as benzene hexachloride (BHC), is a synthetic chemical that exists in four chemical forms called isomers. The different isomers are named according to the position of the hydrogen atoms in the structures. One of these forms, gamma-HCH (or γ-HCH, commonly called Lindane), is produced and used as an insecticide on fruit, vegetables, and forest crops. It is also used as a topical treatment for head and body lice and also for scabies mites which cause contagious skin diseases. γ-HCH is a grayish or brown amorphous solid which vaporizes approximately 100 times faster than DDT. The vapor is colorless and has a slight musty odor. The substance has not been produced in the United States since 1976. However, imported γ-HCH is available in the United States for insecticide use as dust, powder, liquid, and concentrate and also as a lotion, cream, and shampoo to control for scabies mites and head lice.

Technical-grade HCH, a mixture of several chemical forms of HCH, consists of about 10–15% γ-HCH as well as the alpha (α), beta (β), delta (δ), and epsilon (ε) forms of HCH. α-, β-, γ-, and δ-HCH have been found in the soil and surface water at hazardous waste sites. Estimated half-lives in soil from aerobic and anaerobic degradation range from 2.7 to 22.9 years. In the air, the different forms of HCH can be present as a vapor or attached to small particles such as soil and dust; the particles may be removed from the air by rain. γ-HCH can remain in the air for almost 17 weeks depending on moisture in the air and temperature. In soil, sediment, and water, it is broken down to less toxic substances by algae, fungi, and bacteria. Generally, HCH isomers are broken down quickly in water; in natural water samples, γ-HCH does not remain for much longer than 30 days. γ-HCH is not usually found in drinking water. The persistence time of the HCH isomer in soil is not known.

(3) p,p'-Dichlorodiphenyltrichloroethane (DDT)

DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane) is a pesticide that was once widely used to control insects on agricultural crops and insects that carry diseases like malaria and typhus, which resulted in large amounts of DDT being released into the air, soil, and water. DDT is now used in only a few countries to control malaria. Technical-grade DDT is a mixture of three forms: *p,p'*-DDT (85%), *o,p'*-DDT (15%), and *o,o'*-DDT (trace amounts). All of which are white, crystalline, tasteless, and almost odorless solids.

DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) and DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) are breakdown products of DDT which can enter the environment as contaminants. In the past DDD has been used to kill pests, but to a far lesser extent than DDT. One form of DDD (*o,p'*-DDD) has also been used medically to treat cancer of the adrenal gland.

DDT, DDE, and DDD may enter the air upon evaporation from contaminated water and soil. In the air, these compounds will be deposited on land or surface water. Thus, a cycle of evaporation and deposition is produced. As a result, DDT, DDE, and DDD can be carried long distances in the atmosphere. These chemicals have been found in bogs, snow, and animals in the Arctic and Antarctic regions, far from where the chemicals were originally used. DDT, DDE, and DDD may occur in the atmosphere as a vapor or attach to solid particles in the air. Vapor phases of DDT, DDE, and DDD may be broken down in the atmosphere due to reactions caused by sunlight. The half-life of these chemicals in the atmosphere as vapors is calculated to be approximately 1.5-3 days. However, in reality, this half-life estimate is too short to account for the ability of DDT, DDE, and DDD to be carried for long distances in the atmosphere.

It has been found that DDT, DDE, and DDD may last for hundreds of years or longer in soil. These chemicals stick strongly to soil, and generally remain in the surface layers of soil. Some soil particles with attached DDT, DDE, or DDD may enter rivers and lakes through runoff. Only a very small amount, if any, will seep into the ground and migrate into groundwater.

The length of time that DDT will last in soil depends on various factors including temperature, type of soil, and whether the soil is wet. DDT lasts for a much shorter time in the tropics where the chemical evaporates faster and where microorganisms degrade DDT faster. DDT, DDE, and DDD may disappear in less than a year in these warmer climates. In temperate areas, half of the compounds initially present usually disappear in about 5 years. However, in some cases, half will remain for 20, 30, or more years. DDT also disappears faster upon initial entry into the soil. Eventually, the evaporation slows down and the remaining DDT will move into smaller spaces of the soil particles where it is difficult for microorganisms to reach and break down the DDT efficiently. DDT disappears faster when the soil is flooded or wet than when it is dry.

In surface water, DDT binds to particles in the water, then settles and is deposited in the sediment. DDT is taken up by small organisms and fish in the water. It accumulates to high levels in fish and marine mammals (such as seals and whales), reaching levels many thousand times higher than existing in the water. In these animals, the highest levels of DDT are found in their adipose tissues. DDT in the bottom sediment can also be absorbed by some water plants and by the aquatic animals which consume those plants. DDT metabolites can be transported through food webs to top consumers such as humans.

(4) Endosulfan

Endosulfan is a man-made insecticide. It is used for control of a number of insects on food crops such as grains, tea, fruits, and vegetable; on nonfood crops such as tobacco and cotton; and as a wood preservative. Endosulfan is sold as a mixture of two different forms of the same chemical; referred to as alpha- and beta-endosulfan. It is a cream-to-brown-colored solid that may appear crystalline or as flakes; has a distinct odor similar to turpentine; and does not burn. Endosulfan is moderately persistent in the soil environment with a reported average field half-life of 50 days. The two isomers have different degradation times in soil. The half-life for the alpha - isomer is 35 days, and is 150 days for the beta-isomer under neutral conditions. These two isomers will persist longer under more acidic conditions. Endosulfan enters the air, water, and soil during the manufacturing process or when used as a pesticide. Endosulfan is often applied to crops using sprayers. It in the air may travel long

distances before it lands on crops, soil, or water. Endosulfan on crops generally degrades down within a few weeks. Endosulfan is usually found in soil near hazardous waste sites. Endosulfan usually attaches to soil particles and may persist in the soil for several years before being broken down. Endosulfan in soil evaporates into the air where it is broken down. Rainwater can wash endosulfan that is attached to soil particles into surface water. Endosulfan does not dissolve easily in water. Most endosulfan in surface water is attached to soil particles floating in the water or attached to soil at the bottom. Endosulfan does not dissolve easily in water. The small amount of endosulfan that dissolves in water degrades over time. Depending on the conditions in the water, endosulfan may be broken down within 1 day or it may take several months. Some endosulfan in surface water evaporates into the air and is degraded. Because it is water insoluble, low concentrations of endosulfan are usually found in groundwater. Animals inhabiting endosulfan-contaminated water areas can build up endosulfan in their bodies. The amount of endosulfan in their bodies may be several times greater than that in the surrounding water.

(5) Endrin

Endrin is a white, solid, almost odorless substance that was used as a pesticide to control insects, rodents, and birds. Endrin has not been produced or sold for general use in the United States since 1986. Little is known about the properties of endrin aldehyde, an impurity and the breakdown product of endrin, or endrin ketone, which is a product of endrin when it is exposed to sunlight. Endrin does not dissolve very well in water. It has been found in groundwater and surface water but only at very low levels. It is more likely to cling to the bottom sediment of rivers, lakes, and other bodies of water. Endrin is generally not found in the air except when it is applied to fields during agricultural applications. The persistence of endrin in the environment depends highly on local conditions. Some estimates indicate that the half-life of endrin in soil may be up to 12 years. Endrin may also be broken down by exposure to high temperatures (230°C) or sunlight to form primarily endrin ketone and endrin aldehyde. However, the amount of endrin that is broken down to endrin aldehyde or endrin ketone is very small (less than 5%). It is not known what happens to endrin aldehyde or endrin ketone once they are released to the environment.

(6) Heptachlor

Heptachlor is a synthetic chemical that was used in the past for killing insects in homes, buildings, and on food crops. It has not been used for these purposes since 1988 in the United States. There are no natural sources of heptachlor or heptachlor epoxide. Trade names for heptachlor include Heptagran®, Heptamul®, Heptagranox®, Heptamak®, Basaklor®, Drinox®, Soleptax®, Gold Crest H-60®, Termide®, and Velsicol 104®. Heptachlor is both a breakdown product and a component of the pesticide chlordane (approximately 10% by weight). Pure heptachlor is a white powder. Heptachlor smells somewhat like camphor, does not burn easily, does not explode, and does not dissolve easily in water. The half-life of heptachlor in temperate soil is up to 2 years. Technical-grade heptachlor is a tan powder, has a lower level of purity than pure heptachlor, and is the form of heptachlor used most often as a pesticide. Heptachlor epoxide is a breakdown product of heptachlor by microorganisms in the environment, but is not manufactured or used as an insecticide like heptachlor. Like pure heptachlor, heptachlor epoxide is a white powder that does not explode easily. When heptachlor enters animal and human bodies, it will be metabolized to heptachlor epoxide. Heptachlor and heptachlor epoxide are described together in this section because about 20% of heptachlor is changed within hours into heptachlor epoxide in the environment and in the human body.

Heptachlor, or its by-product heptachlor epoxide, can also be found in plants and animals near hazardous waste sites. Although heptachlor is no longer used to kill insects on crops or in homes and buildings, it is still approved by the US EPA for killing fire ants inside power transformers. Heptachlor, or heptachlor epoxide, can also be found in soil and air around buildings treated for termites and in areas where farners have treated seed grains and crops for insects. Heptachlor is able to stick to soil strongly, evaporate into the air slowly, and does not dissolve easily in water. However, heptachlor epoxide dissolves more easily in water than heptachlor does and like heptachlor it sticks to soil. Both heptachlor and heptachlor epoxide are able to travel long distances in the wind after being released on treated fields or manufacturing sites. Heptachlor in the air is eventually deposited on plant leaves. In soil and water, heptachlor is degraded by microorganisms to be more harmful substance such as heptachlor epoxide. Heptachlor epoxide is slowly broken down in

the environment causing it to exist in the soil and the water for many years. Plant roots are able to take up heptachlor present in soil. Animals that consume plants containing heptachlor absorb and convert heptachlor to heptachlor epoxide in their bodies. Both heptachlor and heptachlor epoxide accumulate in fat tissue of fish, cattle, and humans. Some studies show that heptachlor epoxide can exist in fat tissue for 3 years after exposure. Most of the degradation products of heptachlor are thought to be less harmful than heptachlor itself. However, in laboratory studies in animals, heptachlor epoxide is shown to be more harmful than heptachlor.

(7) Methoxychlor

Methoxychlor, also known as DMDT, Marlate®, or Metox®, is a manufactured chemical now used in the United States for controlling insects. Methoxychlor is effective against flies, mosquitos, cockroaches, and a wide variety of other insects. This insecticide is used on agricultural crops and livestock, including animal feed, barns, and grain storage bins. Some pesticide products that consist of methoxychlor are used for controlling insect pests in gardens or on pets.

Pure methoxychlor is a pale-yellow powder that has a slightly fruity or musty odor. It does not readily evaporate into air or dissolve in water. Methoxychlor is very persistent in soil, with a reported representative half-life of approximately 120 days. Pest control operators usually dissolve methoxychlor in a petroleum-based liquid and apply it as a spray, or they mix it with other chemicals and apply it as a dust. Application of methoxychlor as an insect killer accounts for most of the methoxychlor that enters the environment. Since the use of methoxychlor depends on cultivating season, the amount that is released to the environment tends to be greater during pest control periods. Some methoxychlor is released to the environment from chemical plants where methoxychlor is made or from manufacturing sites that formulate products consisting of methoxychlor. A small amount may also be released from hazardous waste sites of where it has been disposed.

Methoxychlor does not occur naturally in the environment. Most methoxychlor enters the environment when it is applied to forests, agricultural crops, and farm animals. It can be applied to forests and crops by aerial spraying causing methoxychlor contamination of nearby land and water. Methoxychlor released into

the air will eventually settle to the ground, rain causing it to settle more quickly, and some may travel long distances before settling.

Once methoxychlor is deposited on the ground, it binds to the soil particles,which can also be blown by the wind or be carried by rainwater or melting snow into rivers or lakes. Most methoxychlor exists in the outermost top layer of soil, but some of the breakdown products may move deeper into the ground. Smaller amounts of methoxychlor in the air may settle directly into rivers, lakes, and other surface water. Once methoxychlor is in the water, it usually binds to sediment or organic matter and settles to the bottom.

Methoxychlor is broken down in the environment by several processes that can be slow and may take months. In the soil, some methoxychlor is broken down by bacteria and other microorganisms, while some may be broken down by reactions to water or materials in the soil. In air and water, methoxychlor can be broken down by sunlight or by reactive chemicals normally present in the air. Some of the breakdown products are capable of producing harmful effects similar to those effects caused by exposure to methoxychlor, for example, estrogenic activity in animals.

Methoxychlor can accumulate in some living organisms including algae, bacteria, snails, clams, and some fish. However, most fish and animals convert methoxychlor into other substances that are rapidly released from their bodies, thus methoxychlor does not usually build up in the food chain.

2.2 Community of Aquatic Organisms

The communities of aquatic organisms which may be affected directly or indirectly by the discharge of OCPRs in this chapter are classified by APHA-AWWA-WPCF (1980) as follows:

(1) Plankton: a community of phytoplankton and zooplankton usually are suspended in water, nonmotile, or insufficiently motile to overcome the transport by currents. In freshwater, they are generally small or microscopic in size.

(2) Periphyton (Aufwuchs): a community of microscopic plants and animals associated with the surface of submersed objects. Some are attached and some move about. Many protozoa and other minute invertebrates and algae that are found in the plankton also occur in the periphyton.

(3) Macrophyton: the larger plants of all types which sometimes attach to the bottom, sometimes are free-floating, sometimes are totally submersed, and sometimes are partly emergent.

(4) Macroinvertebrates: the larger invertebrates which are generally bottom-dwelling organisms (benthos).

(5) Fish: vertebrates that live in water and use its fins and tail to swim.

(6) Amphibians, aquatic reptiles, birds, and mammals: these vertebrates also may be affected by OCPRs, but the discussion is not included in this dissertation.

2.3 Bioaccumulation, Bioconcentration, and Biomagnification in the Food Web

The food web concept (National Research Council [NRC], 2003) defines interactions of interrelated food chain and takes into account species participation in multiple food chains over different trophic levels.

Bioaccumulation is the net accumulation of a contaminant in- and on- an organism from all sources in the environment (Newman, 1998; Ramade, 1992). In this dissertation, it refers to the ratio of concentration in organisms and concentration in sediment (equation 2.1).

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{Concentration in aquatic organisms } (C_n)}{\text{Concentration in sediment } (C_{sediment})} \quad (2.1)$$

Bioconcentration is the different restricted term from bioaccumulation in that the net accumulation of a contaminant in- and on- an organism is from water only (Newman, 1998; Jean-Louis, 1998). It can be estimated from equation 2.2.

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Concentration in aqutic organisms } (C_n)}{\text{Concentration in water } (C_{water})} \quad (2.2)$$

Biomagnification refers to the process by which tissue concentration of bioaccumulation of contaminants increase via the food chain as they pass from one trophic level (e.g. prey) to the next (e.g. predator). Biomagnification results in exposure to higher contaminant levels in top predators of ecosystem (Newman, 1998; NRC, 2003). It can be calculated by equation 2.3.

$$\text{Biomagnification factor (BMF)} = \frac{\text{The concentration from at trophic level n } (C_n)}{\text{The concentration at the next lowest trophic level } (C_{n-1})} \quad (2.3)$$

Walker (1987) reported that the biomagnification of organophosphate insecticides was lower than that of organochlorines. The latter pesticides are well known to be highly persistent in a wide range of organisms (fish, crustaceans, bivalves), due to lipophilic activity and because they exhibit a high bioconcentration factor. Likewise, Ritter *et al*., (1995a; 1995b) reported that biomagnification through the food chain of any organochlorine pesticide is much greater in organisms at the top of the food chain due to their high lipophilicity properties. The study on the limnology (Favari *et al*., 2002) indicated that these pesticides were bioconcentrated 2- to 10-fold from water to algae, 10- to 25-fold in zooplankton, and 8- to 140-fold in fish. This result showed that the bioaccumulation of these contaminants in fish and biomagnification potential in humans are perceived as threats. In addition, Jean-Louis (1998) reported the evaluation of bioconcentration in Table 2.1.

Table 2.1 General evaluation of bioconcentration (Jean-Louis, 1998)

BCF category	Evaluation group	Remarks
<30	I	Low BCF
30-100	II	Average BCF
100-1,000	III	High BCF
>1,000	IV	Very high

2.4 Human Health Risk Assessment

The presentation of risk assessment methods in this dissertation follows the format of the risk assessment process recommended by the National Academy of Sciences [NAS], (1983) (Figure 2.1). Risk assessment can be divided into four major steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization.

Hazard identification is the first step in the risk assessment process. It consists of a review of biological, chemical, and exposure information bearing on the potential for an agent to pose a specific hazard. Hazard identification involves gathering and evaluating data on the types of health effects associated with chemicals of concern under specific exposure conditions (e.g., chronic, acute, airborne, or food borne). A risk assessment might stop with the first step, if no adverse effect is found or if an agency elects to take regulatory action without further analysis, for reasons of policy or statutory mandate.

The second step in the risk assessment process is the evaluation of the dose response dynamics for chemicals of concern. The dose-response dynamic expresses the relationship between exposure and health effects. To evaluate this relationship, the results of human and animal studies are reviewed; the dose-response evaluation may focus on specific types of effects (e.g., developmental, carcinogenic) or be designed to encompass all adverse effects that could occur under any plausible scenario.

The third step in the risk assessment process is exposure assessment. Individual exposure assessments use data on chemical residues in aquatic organism target and human consumption patterns to estimate exposure for hypothetical individuals. Population exposure assessments consider the distributions of exposure in a population. Exposure assessments are then combined with dose response data to determine risk.

The final step in risk assessment is risk characterization, which provides an estimate of the overall individual or population risks. Risk characterization can be used by risk managers to prioritize resource allocation and identify specific at-risk populations; it is also used to establish regulations or guidelines and to estimate individual or population risk.

2.4.1 Hazard Identification

2.4.1.1 Approach for Aquatic Organism Contaminants

The hazard identification step in risk assessment of chemically contaminated fish and shellfish has been refined by EPA (US EPA, 2000b) through careful review

of the chemical characteristics considered to be critical in determining human health risk. These parameters are:

a) High persistence in the aquatic environment
b) High bioaccumulation potential
c) Known sources of contaminant in areas of interest
d) High potential toxicity to humans
e) High concentrations of contaminants in samples of fish or shellfish from areas of interest

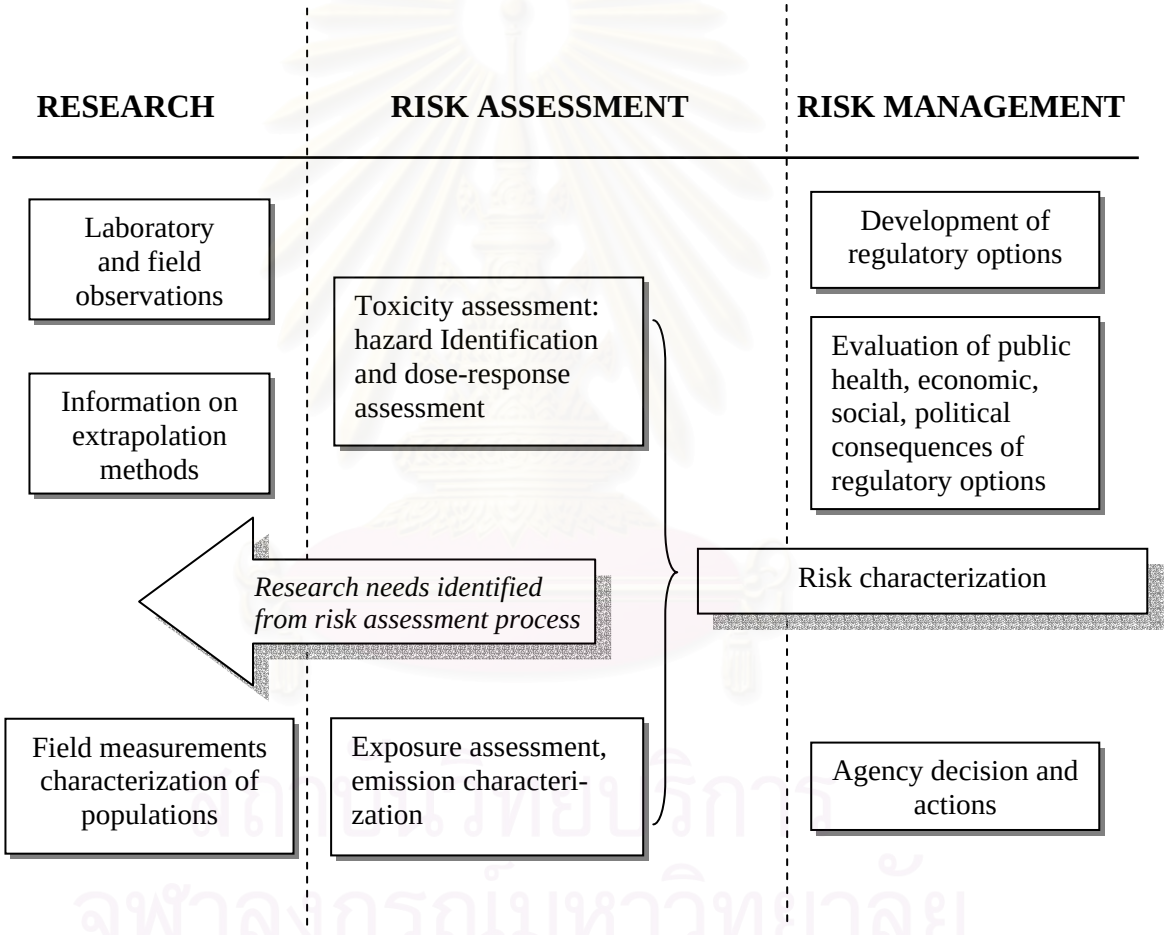


Figure 2.1 Elements or risk assessment and risk management (NAS, 1994)

2.4.1.2 Toxicological Data

(1) Aldrin (Integrated Risk Information System [IRIS], 2007a)

a) Chronic Health Hazard Assessments for Noncarcinogenic Effects

Fitzhugh, Nelson, and Quaife (1964) reported that groups of 24 rats are fed aldrin in the diet for 2 years. Liver lesions characteristic of chlorinated insecticide poisoning are observed at dose levels of 0.5 ppm and greater. These lesions are characterized by enlarged centrilobular hepatic cells, with increased cytoplasmic oxyphilia, and peripheral migration of basophilic granules. Effect and no-effect levels are similar (to those found for rats) for liver effects in dogs after 15 months' exposure to aldrin in the diet. Liver effects were observed at slightly higher doses in several other subchronic-to-chronic rat and dog studies. Short-term exposure to higher doses resulted in mortality for a number of species.

b) Carcinogenicity Assessment for Lifetime Exposure

(a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization:

Classification B2; probable human carcinogen

Basis Orally administered aldrin produced significant increases in tumor responses in three different strains of mice in both males and females. Tumor induction has been observed for structurally related chemicals, including dieldrin, a metabolite.

(b) Human Carcinogenicity Data

Van Raalte (1977) observed the gastric and lymphosarcoma cancer among 166 pesticide manufacturing workers exposed 4 to 19 years and followed from 15 to 20 years. Workers exposed to aldrin and dieldrin did not have an excess risk of cancer.

In a retrospective mortality study, Ditraglia *et al.*, (1981) reported no increased incidence of deaths from cancer among 1155 organochlorine pesticide manufacturing workers (31 observed vs. 37.8 expected, SMR=82).

(2) α-Hexachlorocyclohexane (HCH) (IRIS, 2007b)

a) Carcinogenicity Assessment for Lifetime Exposure

(a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization

Classification B2; probable human carcinogen

Basis Dietary alpha-HCH has been shown to cause increased incidence of liver tumors in five mouse strains and in Wistar rats.

(b) Human Carcinogenicity Data

One case report of a Japanese sanitation employee with acute leukemia was associated with occupational exposure to HCH and DDT (Hoshizaki *et al.*, 1969).

(3) β- Hexachlorocyclohexane (HCH) (IRIS, 2007c)

a) Carcinogenicity Assessment for Lifetime Exposure

(a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization

Classification C; possible human carcinogen

Basis Increases in benign liver tumors in CF1 mice fed beta-HCH

(b) Human Carcinogenicity Data

One case report of a Japanese sanitation employee with acute leukemia was associated with occupational exposure to BHC and DDT (Hoshizaki *et al.*, 1969).

(4) γ- Hexachlorocyclohexane (HCH) (IRIS, 2007h)

a) Chronic Health Hazard Assessments for Noncarcinogenic Effects

Zoecon Corporation (1983) reported that male and female Wistar rats were administered to lindane (99.85%) in the diet. Rats receiving 20 and 100 ppm lindane were observed to have greater-than-control incidence of the following: liver hypertrophy, kidney tubular degeneration, hyaline droplets, tubular distension, interstitial nephritis, and basophilic tubules. The study calculated the dose to be 0.29 mg/kg/day for males and 0.33 mg/kg/day for females, based on measured food intake.

In a 2-year feeding study (Fitzhugh, 1950), Wistar rats were exposed to lindane. Slight liver and kidney damage was found and liver weights were increased by 100 ppm. In a 2-year bioassay (Rivett *et al.*, 1978), beagle dogs were administered lindane in their diet. Treatment-related effects at 100 ppm were increased serum alkaline phosphatase and enlarged dark friable livers. A NOAEL was determined to be 50 ppm (1.6 mg/kg bw/day).

(5) δ- Hexachlorocyclohexane (HCH) (IRIS, 2007d)

a) Carcinogenicity Assessment for Lifetime Exposure

(a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization

Classification D; not classifiable as to human carcinogenicity

(b) Human Carcinogenicity Data

None

(6) p,p'-Dichlorodiphenyl dichloroethane (DDD) (IRIS, 2007m)

a) Carcinogenicity Assessment for Lifetime Exposure

(a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization

Classification B2; probable human carcinogen

Basis based on an increased incidence of lung tumors in male and female mice, liver tumors in male mice and thyroid tumors in male rats. DDD is structurally similar to, and is a known metabolite of DDT, a probable human carcinogen.

(b) Human Carcinogenicity Data

Human epidemiological data are not available for DDD. Evidence for the carcinogenicity in humans of DDT, a structural analog, is based on autopsy studies relating tissue levels of DDT to cancer incidence. Three studies reported that tissue levels of DDT and DDE were higher in cancer victims than in those dying of other diseases (Casarett *et al.*, 1968; Dacre and Jennings, 1970; Wasserman *et al.*, 1976). Studies of occupationally exposed workers and volunteers have been of insufficient duration to determine the carcinogenicity of DDT to humans.

(7) p,p'-Dichlorodiphenyldichloroethylene (DDE) (IRIS, 2007n)

a) Carcinogenicity Assessment for Lifetime Exposure

(a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization

Classification B2; probable human carcinogen

Basis increased incidence of liver tumors including carcinomas in two strains of mice and in hamsters and of thyroid tumors in female rats by diet.

(b) Human Carcinogenicity Data

Human epidemiological data are not available for DDE. Evidence for the carcinogenicity in humans of DDT, a structural analog, is based on autopsy studies relating tissue levels of DDT to cancer incidence. Three studies reported that

tissue levels of DDT and DDE were higher in cancer victims than in those dying of other diseases (Casarett *et al.*, 1968; Dacre and Jennings, 1970; Wasserman *et al.*, 1976). Studies of volunteers and workers occupationally exposed to DDT have been of insufficient duration to determine the carcinogenicity of DDT to humans.

(8) p,p'-Dichlorodiphenyltrichloroethane (DDT) (IRIS, 2007l)

a) Chronic Health Hazard Assessments for Noncarcinogenic Effects

Laug *et al.*, (1950) reported that weanling rats are fed commercial DDT for 15-27 weeks. The diet is prepared by mixing appropriate amounts of DDT in corn oil solution with powdered chow. Females stored more DDT in peripheral fat than did males, but pathologic changes are seen to a greater degree in males. Increasing hepatocellular hypertrophy, especially centrilobularly, increased cytoplasmic oxyphilia, and peripheral basophilic cytoplasmic granules are observed at dose levels of 5 ppm and above. The effect was minimal at 5 ppm (LOAEL) and more pronounced at higher doses. No effects are reported at 1 ppm, the NOEL level use as the basis for the RfD calculation.

DDT fed to rats for 2 years (Fitzhugh, 1948) caused liver lesions at all dose levels (10-800 ppm of diet). A LOAEL of 0.5 mg/kg bw/day was established. Application of a factor of 10 each for uncertainty of estimating a NOEL from a LOAEL, as well as for interspecies conversion and protection of sensitive human subpopulations (1000 total) results in the same RfD level as that calculated from the critical study. As well as, Laug *et al.* (1950) established a LOAEL and a NOEL, with the LOAEL (0.25 mg/kg/day) being the lowest of any observed for this compound.

b) Carcinogenicity Assessment for Lifetime Exposure

(a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization

Classification B2; probable human carcinogen.

Basis Observation of tumors (generally of the liver) in seven studies in various mouse strains and three studies in rats. DDT is structurally similar to other probable carcinogens, such as DDD and DDE.

(b) Human Carcinogenicity Data

The existing epidemiological data are inadequate. Autopsy studies relating tissue levels of DDT to cancer incidence have yielded conflicting results. Three studies reported that tissue levels of DDT and DDE were higher in cancer victims than in those dying of other diseases (Casarett *et al.*, 1968; Dacre and Jennings, 1970; Wasserman *et al.*, 1976). Studies of occupationally exposed workers and volunteers have been of insufficient duration to be useful in assessment of the carcinogenicity of DDT to humans.

(9) Dieldrin (IRIS, 2007e)

a) Chronic Health Hazard Assessments for Noncarcinogenic Effects

Walker *et al.* (1969) administered dieldrin (recrystallized, 99% active ingredient) to rats for 2 years. No effects were seen in various hematological and clinical chemistry parameters. At the end of 2 years, females fed 1.0 and 10.0 ppm (0.05 and 0.5 mg/kg/day) had increased liver weights and liver-to- body weight ratios. Histopathological examinations revealed liver parenchymal cell changes including focal proliferation and focal hyperplasia. These hepatic lesions were considered to be characteristic of exposure to an organochlorine insecticide. The LOAEL was identified as 1.0 ppm (0.005 mg/kg/day) and the NOAEL as 0.1 ppm (0.005 mg/kg/day).

b) Carcinogenicity Assessment for Lifetime Exposure

(a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization

Classification B2; probable human carcinogen

Basis Dieldrin is carcinogenic in seven strains of mice when administered orally. Dieldrin is structurally related to compounds (aldrin, chlordane, heptachlor, heptachlor epoxide, and chlorendic acid) which produce tumors in rodents.

(b) Human Carcinogenicity Data

Two studies of workers exposed to aldrin and to dieldrin reported no increased incidence of cancer. Both studies were limited in their ability to detect an excess of cancer deaths. Van Raalte (1977) observed two cases of cancer (gastric and lymphosarcoma) among 166 pesticide manufacturing workers exposed 4-19 years and followed from 15-20 years. Exposure was not quantified, and workers were also exposed to other organochlorine pesticides (endrin and telodrin).

In a retrospective mortality study, Ditraglia *et al.* (1981) reported no statistically significant excess in deaths from cancer among 1155 organochlorine pesticide manufacturing workers. Exposure was not quantified and workers were also exposed to other chemicals and pesticides (including endrin).

(10) Endrin (IRIS, 2007g)

a) Chronic Health Hazard Assessments for Noncarcinogenic Effects

Velsicol Chemical Corporation (1969) reported that groups of dogs were fed diets containing endrin for 2 years. Dogs receiving 2 or 4 ppm experienced occasional convulsions slightly increased relative liver weights, and mild histopathological effects in the liver (slight vacuolization of hepatic cells). No adverse effects on these parameters or on growth, food consumption, behavior, serum chemistry, urine chemistry or histological appearance of major organs occurred at 1 ppm (NOEL) or less. The 2 ppm level is the LOAEL.

An earlier study (Treon *et al.*, 1955) established a dietary NOEL of 1 ppm for both dogs and rats for long-term feeding (18 months - 2 years). LOAELs of 3 ppm and 5 ppm were reported for dogs and rats, respectively. The primary target organs were the kidney and the liver. Dogs are judged to be more sensitive than rats to long-term exposure to endrin because of the lower food consumption of dogs (than rats)

and because of the much shorter duration of exposure relative to lifetime for dogs as compared to rats.

b) Carcinogenicity Assessment for Lifetime Exposure

(a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization

Classification D; not classifiable as to carcinogenicity for humans

Basis Oral administration of endrin did not produce carcinogenic effects in either sex of two strains of rats and three strains of mice. An NCI bioassay was suggestive of responses in male and female rats although NCI reported a no evidence conclusion. The inadequacies of several of the bioassays call into question the strength of the reported negative findings. These inadequacies and the suggestive responses in the NCI bioassay do not support a Group E classification; rather a Group D classification best reflects the equivocal data.

(b) Human Carcinogenicity Data

Ditraglia *et al.* (1981) conducted a retrospective cohort study to examine the mortality of workers employed in the manufacture of organochlorine pesticides including endrin. No statistically significant excesses or deficits in mortality for any specific cancer site were noted.

(11) Endosulfan (IRIS, 2007f)

a) Chronic Health Hazard Assessments for Noncarcinogenic Effects

Hoechst Celanese Corporation (1989a; 1989b) reported groups of Sprague-Dawley rats were administered endosulfan in the diet for 2 years. No effects of dosing on clinical signs, mortality, food and water consumption, ophthalmological examinations and urinalysis were observed. Mean body weight gains tended to be decreased in both males and females receiving 15 and 75 ppm. No toxicologically important changes in hematology and clinical chemistry parameters were observed. The incidence of bilaterally enlarged kidneys was increased in females. Other findings

in the kidneys were paleness, irregular or uniform cortical scarring and cysts. The incidence of aneurysms of the blood vessels was increased in the high-dose males.

Based on reduced body weight gain in males and females, and increased incidence of marked progressive glomerulonephrosis and blood vessel aneurysms in males, the LEL for systemic toxicity is 75 ppm (Male: 2.9 mg/kg-day; Female: 3.8 mg/kg-day). The NOEL for systemic toxicity is 15 ppm (Male: 0.6 mg/kg-day; Female: 0.7 mg/kg-day).

(12) Heptachlor (IRIS, 2007i)

(a) Chronic Health Hazard Assessments for Noncarcinogenic Effects

Groups of CF strain white rats containing were fed for 2 years with heptachlor in diet. Lesions in the liver were limited to 7 ppm and above and were characteristic of chlorinated hydrocarbons (that is, hepatocellular swelling and peripheral arrangements of the cytoplasmic granules of cells of the central zone of the liver lobules). The NOEL for the lesions was 5 ppm and the LEL was 7 ppm. The NOEL for increased liver-to-body weight for males only was 3 ppm and the LEL was 5 ppm. (Velsicol Chemical Corporation, 1955)

(b) Carcinogenicity Assessment for Lifetime Exposure

a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization

Classification B2; probable human carcinogen

Basis Inadequate human data, but sufficient evidence exist from studies in which benign and malignant liver tumors were induced in three strains of mice of both sexes. Several structurally related compounds are liver carcinogens.

b) Human Carcinogenicity Data

There were 11 case reports involving central nervous system effects, blood dyscrasias, and neuroblastomas in children with pre- or postnatal exposure to

chlordane and heptachlor (IRIS, 2007i). Since no other information was available, no conclusions can be drawn.

There were three epidemiologic studies of workers exposed to chlordane and/or heptachlor. One retrospective cohort study of pesticide applicators was considered inadequate in sample size and duration of follow-up. This study showed marginal statistically significant increased mortality from bladder cancer (3 observed) (IRIS, 2007i).

(13) Heptachlor epoxide (IRIS, 2007j)

a) Chronic Health Hazard Assessments for Noncarcinogenic Effects

Beagle dogs were given diets containing heptachlor epoxide for 60 weeks. Liver-to-body weight ratios were significantly increased in a treatment-related fashion. Effects were noted for both males and females at the LEL of 0.5 ppm. A NOEL was not established (Dow Chemical Company, 1958).

b) Carcinogenicity Assessment for Lifetime Exposure

(a) Evidence for Human Carcinogenicity

Weight-of-Evidence Characterization

Classification B2; probable human carcinogen

Basis Sufficient evidence exists from rodent studies in which liver carcinomas were induced in two strains of mice of both sexes and in CFN female rats. Several structurally related compounds are liver carcinogens.

(b) Human Carcinogenicity Data

There are no published epidemiologic evaluations of heptachlor epoxide. However, there were 11 case reports involving central nervous system effects, blood dyscrasias and neuroblastomas in children with pre-/postnatal exposure to chlordane and heptachlor (IRIS, 2007j). Since no other information was available, no conclusions can be drawn.

The epidemiologic studies of workers exposed to chlordane and/or heptachlor showed marginal statistically significant increased mortality from bladder cancer (IRIS, 2007j). The other retrospective cohort studies were of pesticide manufacturing workers. Neither of them showed any statistically significant increased cancer mortality (IRIS, 2007j; Ditraglia *et al.*, 1981).

(14) Methoxychlor (IRIS, 2007k)

a) Chronic Health Hazard Assessments for Noncarcinogenic Effects

Methoxychlor is considered to have an estrogenic activity. Several recent papers in the open literature have addressed this action of methoxychlor. Kupfer and Bulger (1987) found that both methoxychlor and metabolites have estrogen-like activity with several metabolites having proestrogen activity. They used an in vitro system involving rat liver microsomes and NADPH for a metablizing system with estrogen receptors from immature rat uteri as a detection system.

Gray *et al.* (1989) investigated the effects of methoxychlor on the pubertal development and reproductive function in the male and female rat (Long-Evans hooded) by dosing rats from gestation, weaning, lactation, through puberty with either 25, 50, 100, or 200 mg/kg/day of methoxychlor. In females they found an acceleration of vaginal opening, abnormal estrus cycle, inhibition of luteal function and a blockage of implantation. In males they found an inhibition of somatic growth and accessory gland weight, elevated pituitary and serum prolactin levels, and a suppression of testicular Leydig cell function. Some of these effects occurred at levels as low as 25 mg/kg/day. These observations are consistent with the earlier reports that Methoxychlor mimics estrogen both in vivo and in vitro.

Goldman *et al.* (1986) investigated the subchronic effects of methoxychlor on the rat (Long-Evans hooded) that may be considered an early effect of methoxychlor on the rat reproductive system.

Cummings and Gray (1987) found that methoxychlor affects the decidual cell response of the rat uterus. Long-term exposure to methoxychlor reduced fertility and induced fetotoxicity. Khera *et al.* (1978) on the teratogenicity of methoxychlor found

that treatment of pregnant rats with either technical grade or formulation of methoxychlor produced maternal toxicity in the form of reduced body weight gain at all doses tested (50 to 400 mg/kg/day). A 2-year chronic rat study by Du Pont de Nemours & Co. (1951) reported a systemic NOEL of 100 ppm (5 mg/kg/day); a 2-year chronic study by Hodge *et al.* (1952) reported a systemic NOEL of 200 ppm (10 mg/kg/day).

2.4.2 Dose-response Assessment

2.4.2.1 Carcinogenic Effects

EPA takes a probabilistic approach to estimating carcinogenic risks. Cancer risk is assumed to be proportional to cumulative exposure and, at low exposure levels, may be very small or even zero. EPA assumes that carcinogens do not have "safe" thresholds for exposure; that is, any exposure to a carcinogen may pose some cancer risk. Carcinogenic risk is usually expressed as a cancer potency (CSF) value with units of risk per milligram/kilogram/-day exposure. Risk may also be estimated for specific media. The cancer slope factor is derived from dose-response data obtained in an epidemiological study or a chronic animal bioassay.

2.4.2.2 Noncarcinogenic Effects

Noncarcinogenic effects resulting from multiple exposures occurring over a significant period of time are also termed chronic exposure effects (IRIS, 1999). To protect against chronic toxicity resulting from exposure to contaminants, EPA has developed Reference Doses (RfDs). The RfD is defined as "an estimate (with uncertainty perhaps spanning an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (US EPA, 2000b).

RfDs calculated for chronic noncarcinogenic effects reflect the assumption that, for noncarcinogens and nonmutagens, a threshold exists below which exposure does not cause adverse health effects. This approach is taken for noncarcinogens because it is assumed that, for these types of effects, there are homeostatic, compensating, and adaptive mechanisms that must be overcome before a toxic

endpoint is manifested (IRIS, 1999). RfDs are generally expressed in terms of milligrams of contaminant per kilogram consumer body weight per day (mg/kg-d).

2.4.3 Exposure Assessment (US EPA, 2000b)

2.4.3.1 Individual Exposure Assessment

Individual exposure assessments provide descriptions of the overall, media specific or site-specific exposure of an individual. These may be normative or high (e.g., highly exposed individual) estimates or be based on actual measurement data. Equation 2.4 can be used to calculate the individual exposure to chemical contaminant as following:

$$E_m = \frac{C_m \times CR}{BW} \qquad (2.4)$$

Where:

E_m = individual exposure to chemical contaminant m from ingesting fish or aquatic organisms (mg/kg-d)

C_m = concentration of chemical contaminant m in the edible portion of fish or aquatic organisms (mg/kg)

CR = mean daily consumption rate of fish or aquatic organisms (kg/d)

BW = body weight of an individual consumer (kg)

2.4.4 Risk Characterization

2.4.4.1 Carcinogenic Toxicity

a) Individual Risk

Using cancer slope factor and exposure data in mg/kg-d, cancer risks are calculated using equation 2.5:

Lifetime risk = exposure × cancer potency (2.5)

Where:

exposure = total exposure to a single contaminant from all sources (mg/kg-d)

cancer potency = upper bound of the lifetime cancer risk or cancer slope factor (per mg/kg-day) (shown Table 2.2)

In addition, the lifetime cancer risk equation is the linear approximation that is reasonable for low doses/risks, but that cancer risk cannot exceed 1 and as it approaches 10^{-2}, the exponential form of the equation is needed to make accurate estimates (equation 2.6):

$$\text{Risk} = 1\text{-}e^{\text{-Lifetime risk}} \qquad (2.6)$$

b) Population Risk

The estimated population cancer risk is calculated by multiplying the number of people in an exposure group (with the same exposure) by the lifetime cancer risks calculated from the equation above. The population risk equation is:

$$\text{Population cancer risk} = \text{lifetime risk} \times (\text{size of exposed population}) \qquad (2.7)$$

2.4.4.2 Noncarcinogenic Toxicity

a) Individual Risk

The comparison of exposure to the RfD indicates the degree to which exposure is greater or less than the RfD. The following equation (2.8) expresses this relationship:

$$\text{ratio} = \frac{\text{exposure}}{\text{RfD}} \qquad (2.8)$$

Where:

exposure = total exposure to a single contaminant from all sources (mg/kg-d)

RfD = reference dose or other noncarcinogenic exposure limit (shown in Table 2.2)

When the ratio obtained in the above equation is equal to or greater than 1 (i.e., when exposure exceeds the RfD), the exposed populations may be at risk.

b) Population Risk

The population risk is expressed as the number of individuals with exposure levels greater than the RfD:

noncarcinogenic risk = population with exposure greater than the RfD (2.9)

Table 2.2 Quantitative estimate of noncarcinogenic and carcinogenic risk from oral exposure

Organochlorine pesticides	Noncarcinogenic		Carcinogenic	
	RFD[a] (mg/kg day)	Critical effects	CSF[b] $(mg/kg/day)^{-1}$	Weight-of-Evidence Characterization
Aldrin	3×10^{-5}	Liver	17	B2; probable human carcinogen
α-HCH	not available	not available	6.3	B2; probable human carcinogen
β-HCH	not available	not available	1.8	C; possible human carcinogen
γ-HCH	3×10^{-4}	Liver and kidney toxicity	not available	not available
δ-HCH	not available	not available	none	D; not classifiable as to human carcinogenicity
DDD	not available	not available	2.4×10^{-1}	B2; probable human carcinogen
DDE	not available	not available	3.4×10^{-1}	B2; probable human carcinogen
DDT	5×10^{-4}	Liver lesions	3.4×10^{-1}	B2; probable human carcinogen
Dieldrin	5×10^{-5}	Liver lesions	16	B2; probable human carcinogen
Endrin	3×10^{-4}	Mild histological lesions in liver, occasional convulsions	none	D; not classifiable as to carcinogenicity for humans
Endosulfan	6×10^{-3}	Reduced body weight gain in males and females; increased incidence of marked progressive glomerulonephrosis and blood vessel aneurysms in males	not available	not available
Heptachlor	5×10^{-4}	Liver weight increases increases in males	4.5	B2; probable human carcinogen
Heptachlor epoxide	1.3×10^{-5}	Increased liver-to-body weight ratio in both males and females	9.1	B2; probable human carcinogen
Methoxychlor	5×10^{-3}	Excessive loss of litters	none	D; not classified as to human carcinogenicity

[a] Oral reference dose, and [b] Cancer slope factor were obtained from US EPA's Integrated Risk Information System (IRIS), www.epa.gov/iris/.

CHAPTER III

ORGANOCHLORINE PESTICIDE RESIDUES IN PLANKTON, RANGSIT AGRICULTURAL AREA, CENTRAL THAILAND

3.1 Introduction

Organochlorine pesticides (OCPs) have been used extensively worldwide since the early 1950s. However, due to their persistency in the environment, most of these pesticides are no longer allowed to be used in many countries including Thailand. Because of their low price and broad-killed pests, sometimes farmers illegally use in agricultural fields and they have entered water bodies either directly or indirectly. These pesticides thus still present in the environment (Anat and Paul, 2000; Keithmaleesatti, 2003; Thirakhupt *et al.*, 2006a) and several cause imbalances in biota of the aquatic ecosystem (Favari *et al.*, 2002). In particular, OCPs have often affected non-target organisms and the accumulation by phyto- and zoo- plankton at the base of the aquatic food web may increase and can reach significant concentrations in animals at higher trophic levels through the food web (Robinson *et al*., 1967; DeLorenzo *et al.*, 2002).

In Thailand, there are few studies on organochlorine pesticide residues (OCPRs) contents in freshwater plankton communities. This study was dealing with 2 aims: (1) the study of plankton taxa in material collected by 80 μm plankton net and (2) the investigation of OCPRs concentrations in Khlong 7, Rangsit agricultural area, Pathum Thani Province from June 2006 to February 2007.

3.2 Materials and Methods

3.2.1 Pesticide standard and chemicals

Seventeen organochlorine pesticide standards for α-, γ-, β- and δ-HCH, heptachlor, heptachlor epoxide, aldrin, α-endosulfan, β-endosulfan, endosulfan sulfate 4,4'-DDE, 4,4'-DDD, 4,4'-DDT, dieldrin, endrin, endrin aldehyde, and methoxychlor were obtained from Supelco (Bellefonte, PA, USA). A stock of the standard mixture

containing 17 pesticides was prepared in 99% n-hexane at a concentration of 1,000 ng/mL and stored at -4 ◦C in a refrigerator. Working standard solutions were prepared at the concentration of 0.001–100 ng/mL and then diluted with 99% n-hexane.

Residue analysis solvents such as 95% and 99% n-hexane, dichloromethane, diethyl ether, and petroleum ether were pesticide grade solvents purchased from Labscan Asia Co. Ltd. All chemical reagents were purchased from Fluka Riedel-de Haën i.e. florisil (60-110 mesh) and sodium sulfate anhydrous (granular) which was heated overnight at 300 ◦C. The 500 mg florisil SPE cartidges were purchased from Alltech Associates Inc.

All Pyrex® glassware was well-cleaned with laboratory detergent purchased from EMC-IMEX co., Ltd., then sequentially rinsed with distilled water and acetone. Finally, washed glassware was baked in an oven at 300 ◦C overnight.

3.2.2 Study area and sampling

Rangsit agricultural area is located at the central part of Thailand in Pathum Thani Province. This agricultural area has a man-made irrigation-network-system consisting of 14 sub-canals (Khlong). These sub-canals are divided by Rangsit-Prayulasakdi canal into an upper and lower part. The study area is situated at Khlong 7, a 20-km sub-canal, on the upper part of the irrigation-network-system. Khlong 7 links Raphi Phat canal at the upstream side (14◦12′38.00″N, 100◦45′18.38″E) and Rangsit-Prayulasakdi canal at the downstream side (14◦01′51.25″N, 100◦45′21.25″E) (Figure 3.1).

Field samplings were conducted every 3 months from June 2006 to February 2007. Triplicate samples of plankton were collected from the upper stream (U), middle stream (M), and lower stream (L) of Khlong 7. The plankton samples were taken using a No. 20 net with mesh opening 80 μm (APHA-AWWA-WPCF, 1980). After towing, a 50 mL of plankton was preserved in 2% neutral formalin in glass bottle at room temperature for species identification, and a liter of plankton was contained in polyethylene bottles and maintained below 4 ◦C during transportation and storage until analysis.

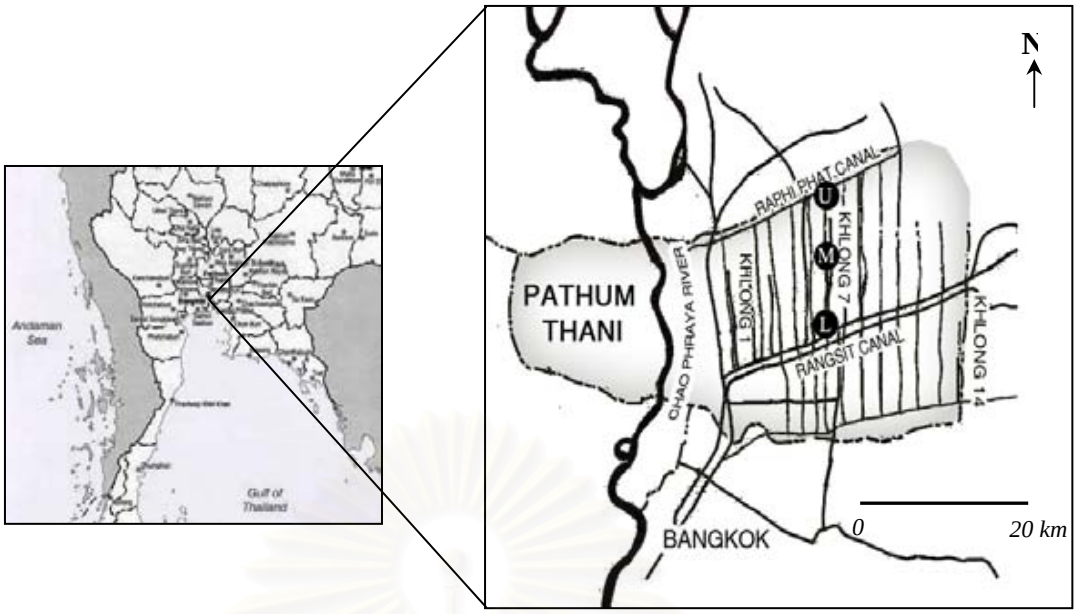


Figure 3.1 Map of Rangsit agricultural area, Pathum Thani Province, Thailand. The sampling stations are at Khlong 7; where (U) =upper stream, (M) = middle stream, and (L) = lower stream

3.2.3 Plankton identification

The preserved planktons were identified to generic level under the conventional light microscope, using keys from Prescott (1978), Bold and Wynne (1985), Taylor (1987), Steidinger and Tangen (1997), Pennak (1989), Dodge and Lee (2000), Graham and Wilcox (2000), John *et al.* (2002), and Pechenik (2005).

3.2.4 Plankton extraction and clean up

The method was modified from DeLorenzo *et al.* (2002). The plankton mass was separated from an aliquot (30 mL) by centrifugation (2500 rpm. for 30 min). The supernatant was decanted. The plankton pellet was then washed with deionized water and recentrifuged twice as before. Afterward, plankton pellet was weighed using 4-digit balance, dissolved in 2 mL methanol and vortexed. An equal amount of hexane was then added and the contents were mixed. After phase separation, a 1-mL aliquot of hexane layer was transfer to clean up. A florisil SPE cartridge was applied for clean up using three fraction eluents: 10 mL of 6%, 15%, and 50% of diethyl ether in petroleum ether, respectively (Caleste Matos Lino and Irene Noronha da Silveira, 1997; Alvin and Lau, 2004). The elution rate was 1 mL/min by gravity. The eluates

were collected in a concentrator tube and volume was reduced to 2 mL under a gentle stream of nitrogen for quantification with GC-μECD (5.2.5).

3.2.5 Gas chromatography analysis

An Agilent 6890N GC equipped with micro Electron Capture Detector (μECD) was used for the quantification. Compound separation was completed using DB-35MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) coated with 35% diphenyl polysiloxane (J&W Scientific). Sample quantification was performed using multiple external standards. A 1.0 μL of sample was injected into the GC on splitless mode with 0.75 min vent delay. The injector and detector temperature were maintained at 260 ˚C and 300 ˚C, respectively. The oven temperature was initially maintained at 100 ºC for 2 min, and then programmed to increase at 12 ºC /min to 280 ºC and held for 10 min. Total run time was calculated to be 27.00 min. For optimum performance, the ultra-high-pure (UHP, 99.999%) helium was used as carrier gas with a flow rate at 2 mL/min linear velocity, and nitrogen (UHP) was set at 60 mL/min as make-up gas.

3.2.6 Quality control

Organochlorine pesticides (OCPs) peaks and retention times were confirmed with DB-1701 fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) coated with 14% cyanopropylphenyl and 86% diphenyl polysiloxane (J&W Scientific). A calibration curve using the external mixed standard of 17 OCPs was performed for each compound to be quantified at concentrations of 1, 2, 5, 10, and 20 ng/mL. Calibration standards were run every 10 samples and all measurements were performed in the ranges of linearity found for each compound. In appendix B, validation data of plankton showed essentially quantitative recovery (mean percent recovery in the range of 84-103% (n=7)) and excellent precision (in the range of 0.20-3.72 %RSD, relative standard deviation) for OCPRs in plankton. The method detection limits (MDLs) were in the range of 0.02-0.49 ng/g wet wt. The limit of detections (LODs) and the limit of quantitations (LOQs) were in the range of 0.001-0.05 ng/mL and 0.002-0.20 ng/mL, respectively, for plankton samples (n=51) taken throughout the sampling period. We considered the method to be reliable to quantify

the concentration of OCPRs in plankton according to AOAC Peer Verified Methods Program (1993).

3.2.7 Statistical analysis

The statistical analysis was performed using SPSS software (Version 12.0). The mean comparison of OCPRs in plankton between wet season (June to November) and dry season (December to May) were determined by using independent samples t-test.

3.3 Results and Discussions

3.3.1 Plankton assemblage

In this study, the planktons, captured in an 80 µm net, were identified only the diversity of phyto- and zoo- plankton taxa in Khlong 7 Rangsit agricultural area, Pathum Thani Province, Thailand from June 2006 to May 2007. Under the conventional light microscope, the results showed that the plankton from three survey sites mainly composed of microphytoplankton and microzooplankton, and mesozooplankton communities. The dominant genera were identified using identified keys listed in Table 3.1. Three genera of microphytoplanktons in the phylum Cyanobacteria were found including of *Merismopedia* sp., *Anabaena* spp., and *Pseudanabaena* sp. In the phylum Euglenophyta, three genera of microphytoplanktons were found; *Euglena* sp., *Phacus* sp., and *Strombomonas* sp. The phylum Dinophyta or dinoflagellates were dominated only by *Gymnodinium* sp. Four genera of microphytoplankton in the phylum Chlorophyta were found such as *Volvox* sp. *Ankistrodesmus* sp., *Tetraedron* sp., and *Pediastrum* spp. Moreover, diatoms in the phylum Bacillariophyta were occasionally found.

For microzooplankton in the phylum Rotifera, *Brachionus* sp. and *Euchlanis* sp. were found. As well as phylum Arthropoda, a number of Cladocera, *Daphnia* sp. (mesozooplankton) and Cyclopoida, cyclopoid copepods (microzooplankton) communities were abundant. Chittapun *et al.* (2007) reported that major zooplankton communities identified from the paddy field in Pathum Thani Province were Rotifera, Cladocera, and Copepoda. These taxa related to this study that may be because paddy fields in Rangsit agricultural area are mainly using water from sub-canals (Khlongs)

for growing rice in each crop causing the circulation of zooplanktons between paddy field and sub-canal. Furthermore, *Daphnia* sp. is mainly recognized as a freshwater cladoceran which is very important components of zooplankton (Martı´nez-Jero´nimo and Martı´nez-Jero´nimo, 2006; Chatmongkolkul and Chantangsi, 2005) and cyclopoid copepods are common found among macrophytes, often swimming around the macrophyte (Sarvala, 1998) such as water hyacinth, water morning glory, and neptunia which are typically distributed in Khlong 7 (Siriwong *et al.*, 2007).

Table 3.1 Phyto- and zoo- plankton taxa found in Khlong 7 Rangsit agricultural area, Pathum Thani Province, Thailand from June 2006 to February 2007

Kingdom Monera (Prokayotes)
Phylum Cyanobacteria Class Cyanophyceae Order Chroococcales Family Chroococcaceae Genus *Merismopedia* Order Nostocales Family Nostocaceae Genus *Anabaena* Family Pseudanabaenaceae Genus *Pseudanabaena* Identification keys are from Bold and Wynne (1985); Graham and Wilcox (2000)
Kingdom Protista (Protists)
Phylum Euglenophyta Class Euglenophyceae Order Euglenales Family Euglenaceae Genus *Euglena*,*Phacus*, and *Strombomonas* Identification keys are from Bold and Wynne (1985); Graham and Wilcox (2000)
Phylum Dinophyta (dinoflagellates) Class Dinophyceae Order Gymnodiniales Family Gymnodiniaceae Genus *Gymnodinium* Identification keys are from Dodge and Lee (2000); Graham and Wilcox (2000); Steidinger and Tangen (1997); Taylor (1987)
Phylum Bacillariophyta (Diatoms) Identification keys are from Hasle and Syvertsen (1997)
Phylum Chlorophyta Class Chlorophyceae Order Volvocales Family Volvocaceae Genus *Volvox* Order Chlorococcales

Family Oocystaceae Genus *Ankistrodesmus* and *Tetraedron* Family Hydrodictyaceae Genus *Pediastrum* Identification keys are from Graham and Wilcox (2000); John *et al.* (2002); Prescott (1978)
Kingdom Animalia
Phylum Rotifera Class Monogononta Order Ploima Family Branchionidae Genus *Brachionus* Family Branchionidae Genus *Euchlanis* Identification keys are from Pennak (1989)
Phylum Arthropoda Class Crustacea Subclass Branchiopoda Order Cladocera: *Daphnia* Subclass Copepoda Order Cyclopoida: Cyclopoid Copepod Identification keys are from Pechenik (2005)

3.3.2 Organochlorine pesticide in plankton

The mean values of OCPRs retained in plankton collected from three survey sites in Khlong 7 were shown in Table 3.2. Low standard error (S.E.) with respect to mean indicated that OCPRs composition did not differ considerably among sites. In Table 3.2 and Figure 3.2 showed that the concentrations of OCPRs during one-year-period contained DDT and derivatives (3.65 ng/g wet wt.) > Σ endosulfan (3.29 ng/g wet wt.) > Σ HCH (1.80 ng/g wet wt.) > Σ heptachlor (1.79 ng/g wet wt.) > aldrin and dieldrin (0.77 ng/g wet wt.) > Endrin and Endrin aldehyde (0.69 ng/g wet wt.) > methoxychlor (0.10 ng/g wet wt.), respectively. The presence of OCPRs in plankton in this agricultural area probably was caused by historical usages and some illegal uses at the present time. Statistical comparisons of OCPRs in plankton between wet- and dry- seasons showed that the residues of Σ HCH, DDT and derivatives, and methoxychlor were higher in wet season than in dry season (independent samples t-test, $p \leq 0.05$). This may be because the heavy rain and runoff may effectively transfer these compounds into canal (Khlong 7) in the wet season. Although Σ HCH, DDT and derivatives, and methoxychlor were banned for few decades ago, but the residues were still found in the soil and other terrestrial environments (Thirakhupt *et al.*,

2006a). Remarkably, Σ endosulfan was still illegally used while this study was performing. It was applied into the paddy field for every crop to mainly kill the golden apple snail (*Pomacea* sp.). However, its residue was not significantly different between wet- and dry- seasons (independent samples t-test, $p \geq 0.05$). Likewise, heptachlor&heptachlor epoxide, aldrin & dieldrin, and endrin & endrin aldehyde which can be found in the soil around buildings and agricultural areas for the elimination of termites and control pests (Thirakhupt *et al.*, 2006a) may be discharged into the canal. They could be then accumulated in planktons resulting in the residues of both wet- and dry- seasons**.**

Table 3.2 The average concentration of OCPRs in plankton (phyto- and zoo-plankton) in the wet season (June to November), dry season (December to May), and one-year study period (June to May) at Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand

OCPs	Average Concentration of OCPRs in Plankton (Mean ± S.E.) (ng/g wet wt.)		
	Wet Season (n=27)	Dry Season (n=24)	One-Year-Period (n=51)
α-BHC	0.57±0.22 [a]	<0.03 [b]	0.30±0.12
γ-BHC	0.88±0.26 [a]	<0.05 [b]	0.47±0.15
β-BHC	0.70±0.11 [a]	0.14±0.07 [b]	0.44±0.08
δ-BHC	1.09±0.39 [a]	0.05±0.05 [b]	0.60±0.22
Σ HCH	3.23±0.50 [a]	0.20±0.10 [b]	1.80±0.34
Heptachlor	2.10±0.84 [a]	0.99±0.39 [a]	1.58±0.48
Heptachlor epoxide	0.24±0.04 [a]	0.18±0.08 [a]	0.21±0.04
Σ Heptachlor	2.34±0.82 [a]	1.17±0.38 [a]	1.79±0.47
Aldrin	0.84±0.26 [a]	0.28±0.09 [a]	0.58±0.15
Dieldrin	0.18±0.06 [a]	0.22±0.11 [a]	0.20±0.06
Aldrin and Dieldrin	1.02±0.26 [a]	0.50±0.13 [a]	0.77±0.15
4,4'-DDE	0.77±0.22 [a]	0.19±0.09 [b]	0.50±0.13
4,4'-DDD	1.46±0.23 [a]	0.20±0.09 [b]	0.86±0.16
4,4'-DDT	3.69±0.54 [a]	0.71±0.26 [b]	2.28±0.37
DDT and derivatives	5.92±0.83 [a]	1.09±0.35 [b]	3.65±0.58
Endosulfan I	0.41±0.12 [a]	<0.003 [b]	0.22±0.07
Endosulfan II	1.12±0.20 [a]	1.62±0.30 [a]	1.36±0.18
Endosulfan sulfate	2.24±0.29 [a]	1.13±0.38 [b]	1.72±0.25
Σ Endosulfan	3.77±0.42 [a]	2.75±0.35 [a]	3.29±0.28
Endrin	0.21±0.09 [a]	0.43±0.30 [a]	0.31±0.15
Endrin aldehyde	0.72±0.14 [a]	<0.01 [b]	0.38±0.09
Endrin and Endrin aldehyde	0.93±0.20 [a]	0.43±0.30 [a]	0.69±0.18
Methoxychlor	0.19±0.06 [a]	<0.01 [b]	0.10±0.03

[a] – statistical comparison between wet and dry season using independent samples t-test, the different letter in the same row indicates the significant difference at $p \leq 0.05$.

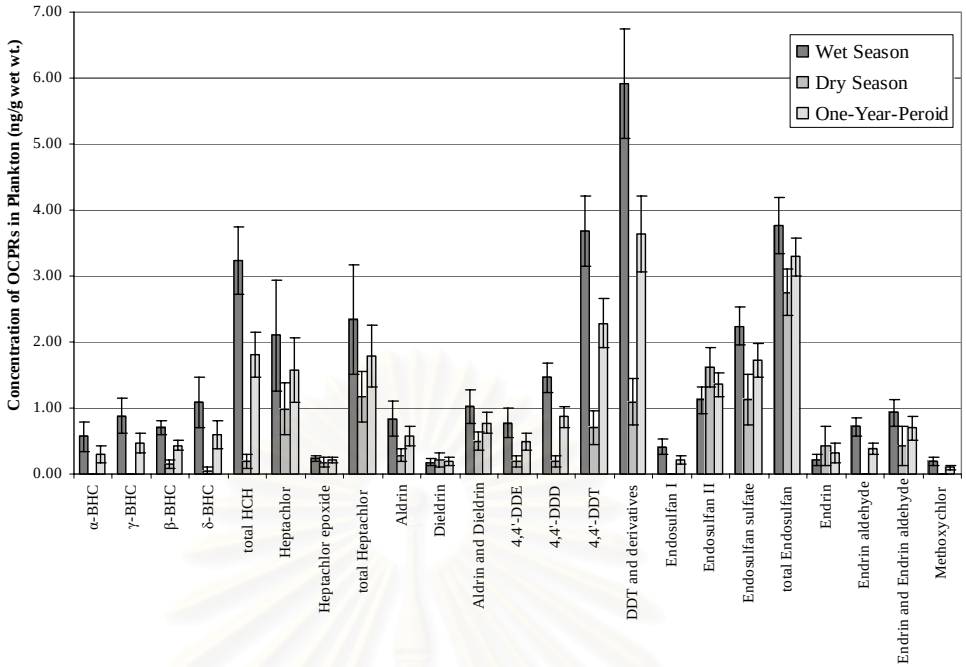


Figure 3.2 Comparison of average concentration of OCPRs in plankton in the wet season, the dry season, and the one-year-period at Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand

3.4 Conclusions

This study revealed that OCPRs are still persisted in plankton communities which are the lowest trophic level of freshwater ecosystem of Khlong 7, Rangsit agricultural area. Although the low concentrations were found, they could be transferred and magnified through the higher trophic level. Therefore, the biomagnification of OCPRs through the food web should be considered in further studies.

CHAPTER IV

ACCUMULATION OF ORGANOCHLORINE PESTICIDE RESIDUES IN AQUATIC PLANTS

4.1 Introduction

Most organochlorine pesticides (OCPs) are persistent organic pollutants (POPs) and are toxic to human and wildlife. OCPs such as DDT, dieldrin, toxaphene, and chlordane have low water solubility, leading to bioaccumulate in fatty tissues and can biomagnify in food chains (Ritter *et al*., 1995; Shengbiao *et al.*, 2006). In Thailand, most OCPs were imported for agricultural and public health purposes in large quantities from 1974 to 1978 (Thirakhupt *et al.*, 2006a). Until 2004, all OCPs were legally banned by the Ministry of Natural Resources and Environment. However, organochlorine pesticides residues (OCPRs) have still existed in all environmental compartments from several agricultural areas and rivers because of their persistent property and illegal used (Thirakhupt *et al.*, 2006a).

It has been concerned that contaminants released into the aquatic environment will establish equilibrium between various compartments in the system. In the aquatic system that has extensive growths of vegetations, there is a possibility that these plants become a sink for organic contaminants. Mark and Klaine (1992) reported that rooted aquatic vascular plants which expose to both overlaying water and sediment are able to absorb chemicals from both of these environments in large amount. Plant lipids are the major factor causing the differences in plant uptake of lipophilic contaminants such as aldrin, dieldrin, heptachlor and heptachlor-epoxide (Chiou *et al*., 2001. Four mechanisms were found to be important in the removal of OCPRs from water by aquatic plants namely (1) rapid sequestration by partitioning to the lipophilic plant cuticles; (2) phytoreduction to less halogenated metabolites; (3) phytooxidation; and (4) assimilation into plant tissues as nonphytotoxic products, presumably produced by covalent binding with the plant tissues (Nzengung and Jeffers, 2001).

The bioaccumulation factor (BAF) and bioconcentration factor (BCF) are used as the criteria for determining and classifying substances that are hazardous to the aquatic environment. Previous studies of BAF and BCF of OCPRs always emphasize

on fish or invertebrates; thus few results of aquatic plants have been reported. Mark and Klaine (1992) reported that BCF for atrazine, lindane, and chlordane found in *Hydrilla verticillata* were 9.62, 38.15, and 1,060.95, respectively in which plant presents the possibility of contaminant redistribution to higher trophic levels through the food chain. Additionally, Mercedes *et al*. (2005) suggested that *Cytisus striatus* which showed high capacity to accumulate ∑ HCH in its leaf and could be used for phytoremediation.

The objective of this study was to investigate the amounts of OCPRs, BAF and BCF in aquatic plants collected from Rangsit agricultural area. Aquatic plants (macrophytons) species studied were: (1) emergent vegetations such as the alligator weed (*Alternanthera philoxeroides*) and the dayflower (*Commelina diffusa*) (2) floating leave plant such as; the red water lily (*Nymphaea lotus*), and (3) free-floating plants such as the water hyacinth (*Eichhornia crassipes*), the water morning glory (*Ipomoea aquatica*), the water primrose (*Ludwigia adscendens*), the neptunia (*Neptunia oleracea*), and the water lettuce (*Pistia stratiotes*).

4.2 Materials and Methods

4.2.1 Pesticide standard and chemicals

Seventeen organochlorine pesticide standards for α-, γ-, β- and δ-HCH, heptachlor, aldrin, heptachlor epoxide, α-endosulfan, β-endosulfan, 4,4'-DDE, 4,4'-DDD, 4,4'-DDT, dieldrin, endrin, endrin aldehyde, endosulfan sulfate, and methoxychlor were obtained from Supelco (Bellefonte, PA, USA). A stock of the standard mixture containing 17 pesticides was prepared in 99% n-hexane at a concentration of 1,000 ng/mL and stored at -4 ◦C in a refrigerator. Working standard solutions were prepared at the concentration of 0.001–100 ng/mL by volume and then diluted with 99% n-hexane.

Residue analysis solvents such as 95% and 99% n-hexane, dichloromethane, diethyl ether, and petroleum ether were pesticide grade solvents purchased from Labscan Asia Co. Ltd. All chemical reagents were purchased from Fluka Riedel-de Haën i.e. florisil (60-110 mesh), anhydrous sodium sulfate (granular), which was heated overnight at 300 ◦C, and copper powder, which was activated with 1% v/v

hydrochloric acid. The 500 mg florisil SPE cartidges were purchased from Alltech Associates Inc.

All Pyrex® glassware was well-cleaned with laboratory detergent purchased from EMC-IMEX co., Ltd., then sequentially rinsed with distilled water and acetone. Finally, washed glassware was baked in an oven at 300 °C overnight.

4.2.2 Study area and sampling

Rangsit agricultural area is located at the central part of Thailand in Pathum Thani Province. This agricultural area has a man-made irrigation-network-system, consisting of 14 sub-canals (Khlong). These sub-canals are divided by Rangsit-Prayulasakdi canal into two parts, the upper part and the lower part. The study area is situated at Khlong 7, a 20-km sub-canal, on the upper part of the irrigation-network-system. Khlong 7 links Raphi Phat canal at the upstream side (14°12′38.00″N, 100 °45′18.38″E) and Rangsit-Prayulasakdi canal at the downstream side (14°01′51.25″N, 100°45′21.25″E) (Figure 4.1).

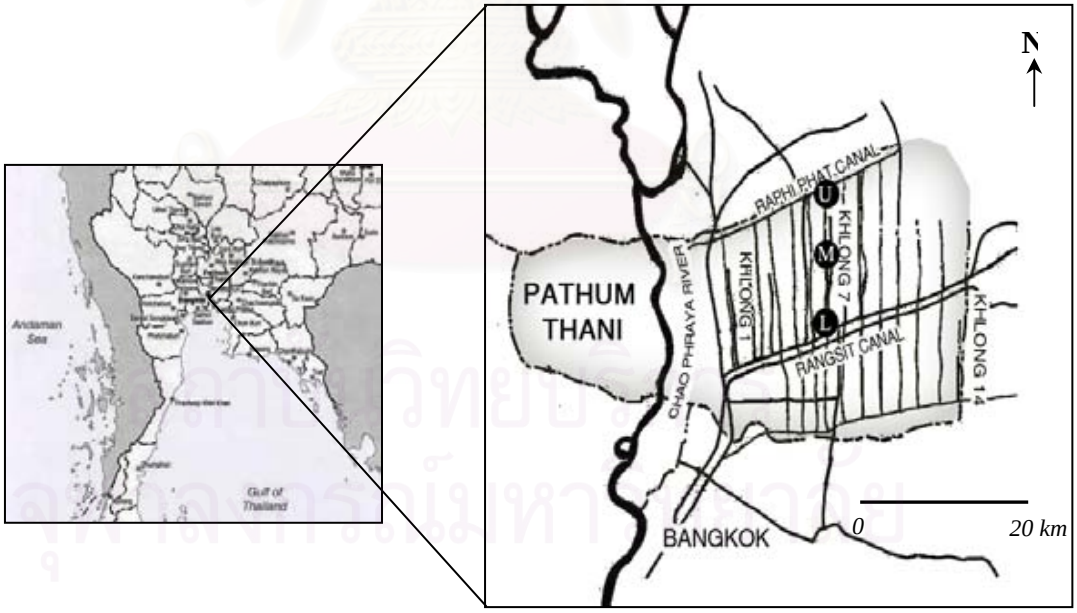


Figure 4.1 Map of Rangsit agricultural area, Pathum Thani Province, Thailand. The sampling stations are at Khlong 7; where (U) = upper stream, (M) = middle stream, and (L) = lower stream

Field samplings were conducted from June 2004 to May 2005. Triplicate samples of water, sediment, and aquatic plants were monthly collected from the upper stream (U), middle stream (M), and lower stream (L) of Khlong 7. Each species of aquatic plants and sediment samples was separately placed in a polyethylene bag and water samples were contained in polyethylene bottles. All samples were maintained below 4 °C during transportation and storage until analysis.

4.2.3 Sample extraction and clean up

4.2.3.1 Extraction of OCPRs in water

Using liquid-liquid extraction (LLE) as described in APHA (1975), the total amount of 800 mL of each surface water sample was filtered with Whatman® filter paper (i.d. 70 mm) then poured into 2-L separatory funnel. For the first LLE, the mixture of 100 mL n- hexane and dichloromethane (1:1 v/v) was added and shaken vigorously for 2 min before 2-phase separated at least 10 min. The water-phase was drained from the separatory funnel into a 1,000 mL beaker, and carefully poured the organic phase to a glass funnel containing a 20 g anhydrous sodium sulfate through a 200-mL concentrator tube. Following the second and third LLE, water-phase was poured back into the separatory funnel to re-extract with 50 mL of the same solvent mixtures. The extract was concentrated to the volume of 2 mL under a gentle stream of nitrogen using Turbo Vap® evaporator, and then analyzed with GC-μECD (4.2.4).

4.2.3.2 Extraction of OCPRs in sediment

Each sediment sample had been well-mixed and dried in a circulating air at the room temperature without sunlight exposure for 3-4 days. Dried sample was ground and sieved (500 μm) to remove stones and shells (Pridmore *et al*., 1992). Using accelerated solvent extractor (ASE, Dionex Canada Ltd. Oakville, ON, Canada), 5 g of the sediment sample was mixed with 5 g anhydrous sodium sulfate (1:1 w/w) and placed into a 34-mL ASE-vessel, then extracted with 1:1 v/v 95% n-hexane: dichloromethane. The sample was preheated for 5 min and extracted at 100°C with pressure 1,500 psi for 10 min. Finally, sample was purged with nitrogen for 60 sec.

To remove sulfur contamination as previously described in Pan *et al.* (2004), the elute was cleaned up with chromatographic column by packing 6 g of florisil layer between 2 g of activated copper powder and 10 g of anhydrous sodium sulfate layer. Three fractions of eluents were used specifically: 50 mL of 6%, 15%, and 50% of diethyl ether in petroleum ether, respectively. The elution rate was 5 mL/min by gravity. The eluates were collected in a concentrator tube and reduced the volume to 2 mL under a gentle stream of nitrogen for quantification with GC-μECD (4.2.4).

4.2.3.3 Extraction of OCPRs in aquatic plants

The mixture of 1:1 v/v 95% n-hexane: dichloromethane was used as solvent for ASE with the operating conditions as same as sediment extraction. A 5 g of blended aquatic plant was mixed with 20 g anhydrous sodium sulfate containing in the ASE-vessel. Following pigment removal (Caleste Matos Lino and Irene Noronha da Silveira, 1997; Alvin and Lau, 2004), a florisil solid phase extraction (SPE) was applied for clean up using three fraction eluents: 10 mL of 6%, 15%, and 50% of diethyl ether in petroleum ether, respectively. The elution rate was 1 mL/min by gravity through the florisil SPE cartridge. The eluates were collected in a concentrator tube and reduced the volume to 2 mL under a gentle stream of nitrogen for quantification with GC-μECD (4.2.4).

4.2.4 Gas chromatography analysis

An Agilent 6890N GC equipped with micro Electron Capture Detector (μECD) was used for the determination. By using DB-35MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) coated with 35% diphenyl polysiloxane (J&W Scientific) to analyze OCPRs and DB-1701 fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) coated with 14% cyanopropylphenyl and 86% diphenyl polysiloxane (J&W Scientific) to confirm OCPs peaks and retention times. A 1.0 μL of sample was injected into the GC on splitless mode with 0.75 min vent delay. The injector and detector temperature were maintained at 260 °C and 300 °C, respectively. The oven temperature was initially maintained at 100 ºC for 2 min, and then programmed to increase at 12 ºC /min to 280 ºC and held for 10 min. Total run time was calculated to be 27.00 min. For optimum performance, the ultra-high-pure (UHP, 99.999%) of helium was used as carrier gas

with flow rate at 2 mL/min linear velocity, and nitrogen (UHP) was set at 60 mL/min as make-up gas.

4.2.5 Quality control

Organochlorine pesticides (OCPs) were identified by peaks and retention times on a capillary column DB-35MS and confirmed with DB-1701 as mentioned earlier. Calibration curve using the external mixed standard of 17 OCPs was performed for each compound to be quantified at concentrations of 5, 10, 20, 50, and 100 ng/mL. All measurements were performed in the ranges of linearity found for each compound. Following the AOAC Peer Verified Methods Program (1993), the limit of detection (LOD) of OCPs were in the range of 0.001-0.05 ng/mL, the limit of quantitation (LOQ) of OCPs were in the range of 0.002-0.20 ng/mL and the method detection limit (MDL) of OCPs for water, sediment, and aquatic plants were in the range of 0.001-0.01 ng/mL, 0.66-1.46 ng/g dry wt., and 0.63-3.96 ng/g wet wt., respectively. For accuracy and precision checking, the recoveries of OCPs for water, sediment, and aquatic plants were in the range of 71-120 %, 75-93 %, and 61-116 %, respectively. The relative standard deviations (RSD) of OCPs for water, sediment, and aquatic plants were in the range of 3-12 %, 2-4 %, and 3-12 %, respectively. Therefore, we considered the method to be reliable to quantify the concentration of OCPRs in all matrices.

4.2.6 Quantification of bioaccumulation and bioconcentration

Bioaccumulation is the net accumulation of a contaminant in- and on- an organism from all sources in the environment (Newman, 1998). In this paper, it refers to the ratio of concentration in organism and concentration in sediment (equation 1).

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{Concentration in aquatic plants}}{\text{Concentration in sediment}} \quad (4.1)$$

Bioconcentration is the different restricted term from bioaccumulation in that the net accumulation of a contaminant in- and on- an organism is from water only (Newman, 1998). It can be estimated from equation 2.

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Concentration in aqutic plants}}{\text{Concentration in water}} \qquad (4.2)$$

4.2.7 Statistical analysis

Using SPSS software for window, multiple mean comparisons of OCPRs in aquatic plants were conducted using ANOVA with LSD (equal variances assumed) and with Tamhane's T2 (equal variances not assumed).

4.3 Results and Discussions

4.3.1 Organochlorine pesticides in water, sediment, and aquatic plants

Table 4.1 illustrates the concentration means with standard error (S.E.) values of OCPRs in water (ng/mL), sediment (ng/g), and aquatic plants (ng/g) from Khlong 7 Rangsit agricultural area from June 2004 to May 2005. All 7 groups of OCPRs including of ∑ hexachlorocyclohexane (α-, γ-, β- and δ-HCH), heptachlor & heptachlor epoxide, aldrin & dieldrin, DDT & derivatives, ∑ endosulfan (α-, β- and –sulfate), endrin & endrin aldehyde, and methoxychlor were found. The concentration of OCPRs in water was compared with the Criteria Maximum Concentration (CMC) and the Criterion Continuous Concentration (CCC) reported in the National Recommended Water Quality Criteria (US EPA, 2006). All OCPRs values did not exceed the CMC recommendation limits. However, 6 OCPRs except endrin & endrin aldehyde were over the CCC criteria. All OCPRs mean values in water were in the range of those samples collected from other agricultural areas in Thailand on 1997 (Anat and Paul, 2000). ∑ endosulfan (0.08 ng/mL) was the highest concentration found in water probably because endosulfan has been illegally used to control the apple snails and various pests in paddy fields. Furthermore, ∑ HCH, DDT and derivatives, and heptachlor & heptachlor epoxide were still found because they were banned recently (Thirakhupt *et al.*, 2006a). Aldrin & dieldrin (0.007 ng/mL) and endrin & endrin aldehyde (0.007 ng/mL) were found in low concentration because they had been banned since 1980s. Noticeably, methoxychlor was not imported for usage in Thailand, but low concentration of 0.001 ng/mL was detected. Methoxychlor may be transported from neighboring countries through the atmosphere then gradually deposited into the river at the higher latitude (Ruey-An *et al.*, 2002) or directly

precipitated into this area. In the sediment, the concentrations of heptachlor & heptachlor epoxide (14.67 ng/g dry wt.) and DDT and derivatives (12.05 ng/g dry wt.) were high because the octanol-water partition coefficients (K_{ow}) of heptachlor & heptachlor epoxide (log K_{ow}= 4.4-5.5) and DDT and derivatives (log K_{ow}=5.5-6.19) are greater than HCH (log K_{ow}= 3.8) and endosulfan (log K_{ow}= 3.55-3.62). As a result, those compounds were better trapped by sediment particles than dissolved in water. However, DDT and derivatives residue found in sediment from Victoria harbor, Hong Kong was 10.2 ng/g dry wt. (Hong *et al*., 1999) and its concentration in sediment of Macao estuary, China was 12.8 ng/g dry wt (Zhang *et al*., 1999), as similar as shown in Table 4.1 (12.05 ng/g dry wt.). In addition, the residue of ∑ HCH from the northern coast, Vietnam (Nhan *et al*., 1999) reported at 8.53 ng/g dry wt in sediment related to the result of ∑ HCH (9.36 ng/g dry wt.) in Table 4.1.

Among 8 aquatic plants, high concentrations of ∑ HCH (36.49 ng/g wet wt), DDT and derivatives (19.61 ng/g wet wt), and ∑ endosulfan (14.03 ng/g wet wt) were found in *Neptunia oleracea*. The highest concentration of aldrin & dieldrin (3.22 ng/g wet wt) and endrin & endrin aldehyde (5.34 ng/g wet wt) were detected in *Ludwigia adscendens*. In *Alternanthera philoxeroides*, the concentration of heptachlor & heptachlor epoxide (6.00 ng/g wet wt.) was higher than in other species. Methoxyclor (0.79 ng/g wet wt.) was high in *Eichhornia crassipes*. Statistically, *Neptunia oleracea* had the highest DDT and derivatives when compared to other aquatic plants (LSD, $p \leq 0.05$). The concentrations of ∑ endosulfan found in *Neptunia oleracea*, *Ipomoea aquatica, and Alternanthera philoxeroides* were not significantly different (Tamhane's T2, $p > 0.05$). For all plant species, heptachlor & heptachlor epoxide concentrations were not significantly different (Tamhane's T2, $p > 0.05$).

Pimpan *et al*. (1995) reported higher amount of dieldrin in aquatic plants*; Eichhornia crassipes* (0.012 mg/kg wet wt.), *Pistia stratiotes* (0.010 mg/kg wet wt.), and *Nymphaea lotus* (0.008 mg/kg wet wt.) collected from Bung Boraphed reservoir in the central part of Thailand in 1989. The concentration of dieldrin was much greater than of aldrin&dieldrin in this study. It may be because farmers had used this insecticide extensively to kill crop pests and termites until it was banned in 1988. Moreover, plants could take up dieldrin and stored it in their leaves and roots (Thirakhupt *et al.*, 2006a).

In particular, the residue of heptachlor & heptachlor epoxide in edible aquatic plant species such as *Neptunia oleracea* (4.19 ng/g wet wt.) and *Ipomoea aquatica* (5.39 ng/g wet wt.) did not exceed the maximum residue limit (MRL) for general vegetable of European Union (EU) (≤ 0.01 mg/kg), CODEX/WHO (≤ 0.05 mg/kg), and Thailand (≤ 0.1 mg/kg). Furthermore, the residue of ∑ endosulfan in *Neptunia oleracea* (14.03 ng/g wet wt.) and *Ipomoea aquatica* (13.79 ng/g wet wt.) exceeded the MRL for general vegetable of European Union (EU) (≤ 0.01 mg/kg). In contrast, the residues of DDT and derivatives in both species did not exceed the MRL of European Union (EU) (≤ 0.1 mg/kg) and Thailand (≤ 2.0 mg/kg).

Table 4.1 The concentration of organochlorine pesticide residues (mean ± S.E.) in water (ng/mL), sediment (ng/g dry wt.), and aquatic plants (ng/g wet wt.) from Khlong 7, Rangsit agricultural area from June 2004 to May 2005

Environment Compartments	n	Concentration of OCPRs (mean ± S.E.) (ppb)						
		∑ HCH	Heptachlor & Heptachlor epoxide	Aldrin & Dieldrin	DDT & derivatives	∑ Endosulfan	Endrin & Endrin aldehyde	Methoxychlor
Water*	108	0.014 ± 0.002	0.007 ± 0.001	0.007 ± 0.001	0.019 ± 0.001	0.082 ± 0.01	0.005 ± 0.001	0.001 ± 0.003
Sediment♣	108	9.36 ± 0.27	14.67 ± 0.48	2.97 ± 0.23	12.05 ± 0.30	6.36 ± 0.25	0.78 ± 0.12	0.04 ± 0.02
Aquatic plants (macrophytons)†, §								
Eichhornia crassipes (Hart.) Solms	84	5.44 ± 0.32^{a}	5.90 ± 0.21^{a}	2.13 ± 0.19^{ab}	9.25 ± 0.55^{ab}	7.91 ± 0.49^{a}	1.49 ± 0.26^{a}	0.79 ± 0.08^{d}
Alternanthera philoxeroides (Mart.) Griseb	30	8.51 ± 0.53^{bcd}	6.00 ± 0.34^{a}	2.42 ± 0.30^{a}	7.99 ± 1.16^{ab}	13.35 ± 0.85^{b}	0.15 ± 0.09^{b}	0.26 ± 0.09^{ab}
Ipomoea aquatica Forssk.	42	7.59 ± 0.64^{ab}	5.39 ± 0.27^{a}	2.88 ± 0.74^{a}	6.94 ± 0.60^{a}	13.79 ± 1.47^{b}	0.76 ± 0.21^{a}	0.34 ± 0.11^{ab}
Nymphaea lotus L.	57	12.76 ± 1.36^{c}	5.64 ± 0.32^{a}	2.14 ± 0.25^{ab}	8.86 ± 1.32^{ab}	8.22 ± 0.86^{a}	0.73 ± 0.21^{a}	0.09 ± 0.02^{b}
Pistia stratiotes L.	33	10.59 ± 1.11^{bc}	5.48 ± 0.30^{a}	2.53 ± 0.41^{a}	11.12 ± 1.91^{b}	13.01 ± 1.70^{a}	2.14 ± 0.50^{a}	0.10 ± 0.04^{bc}
Ludwigia adscendens (L.) Hara	9	9.28 ± 0.93^{abc}	4.87 ± 1.00^{a}	3.22 ± 0.74^{a}	9.12 ± 0.60^{ab}	9.99 ± 1.01^{a}	5.34 ± 1.34^{a}	0.55 ± 0.14^{bcd}
Commelina diffusa Burm. f.	12	6.33 ± 0.59^{ad}	5.80 ± 0.23^{a}	0.72 ± 0.11^{b}	8.17 ± 1.42^{ab}	9.53 ± 1.22^{a}	0.14 ± 0.09^{b}	0.13 ± 0.07^{ab}
Neptunia oleracea Lour.	6	36.49 ± 6.79^{abc}	4.19 ± 1.16^{a}	2.90 ± 0.23^{ab}	19.61 ± 2.38^{c}	14.03 ± 0.29^{b}	< 0.002♠^{b}	< 0.005♠^{ac}

*ng/mL, ♣ ng/g dry wt., and †ng/g wet wt.

§ The mean concentrations in each column with the different letter are significantly different at $p \leq 0.05$

♠detection limits

4.3.2 Bioaccumulation and bioconcentration

Table 4.2 shows bioaccumulation factors (BAF) and bioconcentration factors (BCF) of OCPRs in aquatic plants from Khlong 7, Rangsit agricultural area from June 2004 to May 2005. For emergent vegetations, *Alternanthera philoxeroides* showed the maximum BAF for methoxychlor (BAF=7) and ∑ endosulfan (BAF=2.09×10^2). Likewise, floating leave plant, *Nymphaea lotus* presented the maximum BAF for methoxychlor (BAF=3). For free-floating plants, the maximum BCF for heptachlor & heptachlor epoxide in *Eichhornia crassipes*, *Ipomoea aquatica*, and *Pistia stratiotes* were 9×10^2, 8×10^2, and 8×10^2 times of the water concentration, respectively. BCF for endrin & endrin aldehyde in *Ludwigia adscendens* was 1×10^2. Similarly, *Neptunia oleracea* showed the highest BCF for ∑ HCH (BCF=2×10^2). Xia *et al*. (2002) reported that *Eichornia crassipes* could be used for phytoremediation of several pesticides such as ethion, dicofol (related in structure to DDT) and cyhalothrin. In this study, *Eichornia crassipes* can be used in phytoremediation for heptachlor & heptachlor epoxide due to its the highest BCF value among free-floating plants.

The BAFs of *Alternanthera philoxeroides*, *Commelina diffusa*, and *Nymphaea lotus* were 1 for ∑ HCH and DDT & derivatives, indicating that they have potential for phytoremediation in sediment as previously mentioned in Table 4.1. For high residues of DDT & derivatives and ∑ HCH in the surface water, *Neptunia oleracea* efficiently absorbed these residues with BCF equal to 1.0×10^3, and 2.5×10^3, respectively and may be used for remediation process. *Neptunia oleracea* (BCF=1.7×10^2) and *Ipomoea aquatica* (BCF=1.7×10^2) were also suitable for the elimination of ∑ endosulfan residues in surface water.

Table 4.2 Bioaccumulation factors (BAF) and bioconcentration factors (BCF) of OCPRs in aquatic plants between environmental compartments from Khlong 7, Rangsit agricultural area from June 2004 to May 2005

Scientific name	Common name	∑ HCH	Heptachlor & Heptachlor epoxide	Aldrin & Dieldrin	DDT & derivatives	∑ Endosulfan	Endrin & Endrin aldehyde	Methoxychlor
Bioaccumulation factor (BAF) *								
Alternanthera philoxeroides (Mart.) Griseb	Alligator weed	0.909	0.409	0.817	0.663	2.09	0.19	7
Commelina diffusa Burm. f.	Climbing dayflower	1.36	0.385	0.722	0.736	1.29	0.94	2
Nymphaea lotus L.	Water lily	0.677	0.396	0.24	0.678	1.49	0.18	3
Bioconcentration factor (BCF) *								
Eichhornia crassipes (Hart.) Solms	Water hyacinth	3.8×10^2	9×10^2	3×10^2	4.8×10^2	9.6×10^2	3×10^2	8×10^2
Ipomoea aquatica Forssk.	Swamp morning-glory	5.3×10^2	8×10^2	4×10^2	3.6×10^2	1.7×10^2	2×10^2	3×10^2
Pistia stratiotes L.	Water lettuce	7.4×10^2	8×10^2	4×10^2	5.8×10^2	1.6×10^2	4×10^2	1×10^2
Ludwigia adscendens (L.) Hara	Water primrose	6.5×10^2	7×10^2	4×10^2	4.7×10^2	1.2×10^2	1×10^3	5×10^2
Neptunia oleracea Lour.	Neptunia	2.5×10^3	6×10^2	4×10^2	1.0×10^3	1.7×10^2	< 0.4	< 5

* BAF and BCF are based on whole body (WB) of aquatic plants measurements and calculated on a wet weight basis.

4.4 Conclusions

This study provides the recent data of OCPRs in water and sediment of Rangsit agricultural area, a very important cultivation area of the central plain of Thailand. Furthermore, the results of BAF and BCF showed that 7 groups of OCPs including hexachlorocyclohexane (α-, γ-, β- and δ-HCH), heptachlor & heptachlor epoxide, aldrin & dieldrin, DDT & derivatives, endosulfan (α-, β- and -sulfate), endrin & endrin aldehyde, and methoxychlor could be accumulated in all aquatic plant species. Some plant species have the potential for phytoremediation. Nevertheless, we suggest that the risk assessment of ∑ HCH, ∑ endosulfan, and heptachlor & heptachlor epoxide for vegetable consumption is required especially in *Neptunia oleracea* and *Ipomoea aquatica* for the reason that both species are frequently consumed by local residents.

CHAPTER V

BIOMAGNIFICATION OF ORGANOCHLORINE PESTICIDES IN AQUATIC FOOD WEB OF RANGSIT AGRICULTURAL AREA, CENTRAL THAILAND

5.1 Introduction

In Thailand, organochlorine pesticides (OCPs) had been heavily used for agricultural and public health purposes started in the 1950s and reached maximum in the 1970s through the 1990s. Although most OCPs have been banned for nearly two decades, their residues are still found in all aquatic ecosystem matrices such as water, sediment, and aquatic organisms (Anat and Paul, 2000; Thirakhupt *et al.*, 2006a). OCPs are stable organic compounds which have very low water solubility and high lipophilicity. Some of them are highly persistent in their original forms or as stable metabolites. These residues are still slowly releasing into aquatic and terrestrial food chains and can reach significant concentrations in animals at higher trophic levels (Robinson *et al*., 1967; Keithmaleesatti, 2003). Examples include DDT, chlordane, aldrin, dieldrin, and heptachlor.

To concern about OCPs effects on nonhuman species, DDT and DDD were documented that they could accumulate in wildlife resulting in direct toxicity and sub-lethal effects (Newman, 1998). Furthermore, high OCPs concentrations in an organism may impair its endocrine, reproductive and nervous systems (Borgå *et al*, 2001). As evidenced by DDT poisoning of bird shown in *Silent Spring*, the extraordinary book by Carson (1962), the transfer of contaminant through trophic webs can have undesirable consequences to top predators. DDT displays biomagnification, an increase in contaminant concentration from one trophic level to the next due to accumulation from food (Keithmaleesatti *et al.*, 2006).

This study aimed to investigate the biomagnifications (BMFs) of organochlorine pesticides in the selected predators and preys in the food web of aquatic ecosystem at Khlong 7 (canal), Rangsit Agricultural Area. The food relationship was investigated based on foraging behavior observation in laboratory

aquarium of Khlong 7 fish, stomach analysis, and using literatures (Nelson, 1976; Rainboth, 1996; Monkolprasit *et al*, 1997; Vidthayanon, 2002; Vidthayanon, 2004), Fifteen common organisms were selected to represent the foraging behavior in the food web; (1) 2 producers; *Eichhornia crassipes* and plankton (phyto- and zoo-plankton) (2) an herbivore; *Trichogaster microlepis* (3) 3 omnivores; *Trichogaster trichopterus*, *Oreochromis niloticus*, and *Puntius gonionotus* (4) 6 carnivores; *Channa striatus, Oxyeleotris marmoratus*, *Macrognathus siamensis*, *Parambassis siamensis*, *Anabas testudineus*, and *Pristolepis fasciatus*, and (5) 3 detritivores; *Macrobrachium lanchesteri*, *Pomacea* sp., and *Filopaludina mertensi.* The bioconcentration factor (BCF), bioaccumulation factor (BAF), and biomagnification factor (BMF) of ∑ hexachlorocyclohexane (α-, γ-, β- and δ-HCH), heptachlor & heptachlor epoxide, DDT & derivatives, and ∑ endosulfan (α-, β- and - sulfate) were calculated through out the food chain from the lowest trophic level to highest trophic level. We expected that the OCPs burden in prey and predators is still existed and elevated in organisms although OCPs was banned.

5.2 Materials and Methods

5.2.1 Pesticide standard and chemicals

Seventeen organochlorine pesticide standards for α-, γ-, β- and δ-HCH, heptachlor, heptachlor epoxide, aldrin, α-endosulfan, β-endosulfan, endosulfan sulfate 4,4'-DDE, 4,4'-DDD, 4,4'-DDT, dieldrin, endrin, endrin aldehyde, and methoxychlor were obtained from Supelco (Bellefonte, PA, USA). A stock of the standard mixture containing 17 pesticides was prepared in 99% n-hexane at a concentration of 1,000 ng/mL and stored at -4 ◦C in a refrigerator. Working standard solutions were prepared at the concentration of 0.001–100 ng/mL by volume and then diluted with 99% n-hexane.

Residue analysis solvents such as 95% and 99% n-hexane, dichloromethane, diethyl ether, and petroleum ether were pesticide grade solvents purchased from Labscan Asia Co. Ltd. All chemical reagents were purchased from Fluka Riedel-de Haën i.e. florisil (60-110 mesh) and anhydrous sodium sulfate (granular), which was heated overnight at 300 ◦C, and copper powder, which was activated with 1% v/v

hydrochloric acid. The 500 mg florisil SPE cartidges were purchased from Alltech Associates Inc.

All Pyrex® glassware was well-cleaned with laboratory detergent purchased from EMC-IMEX co., Ltd., then sequentially rinsed with distilled water and acetone. Finally, washed glassware was baked in an oven at 300 °C overnight.

5.2.2 Study area and sampling

Rangsit agricultural area is located at the central part of Thailand in Pathum Thani Province. This agricultural area has a man-made irrigation-network-system consisting of 14 sub-canals (Khlong). These sub-canals are divided by Rangsit-Prayulasakdi canal into an upper and lower part. The study area is situated at Khlong 7, a 20-km sub-canal, on the upper part of the irrigation-network-system. Khlong 7 links Raphi Phat canal at the upstream side (14°12′38.00″N, 100°45′18.38″E) and Rangsit-Prayulasakdi canal at the downstream side (14°01′51.25″N, 100°45′21.25″E) (Figure 5.1).

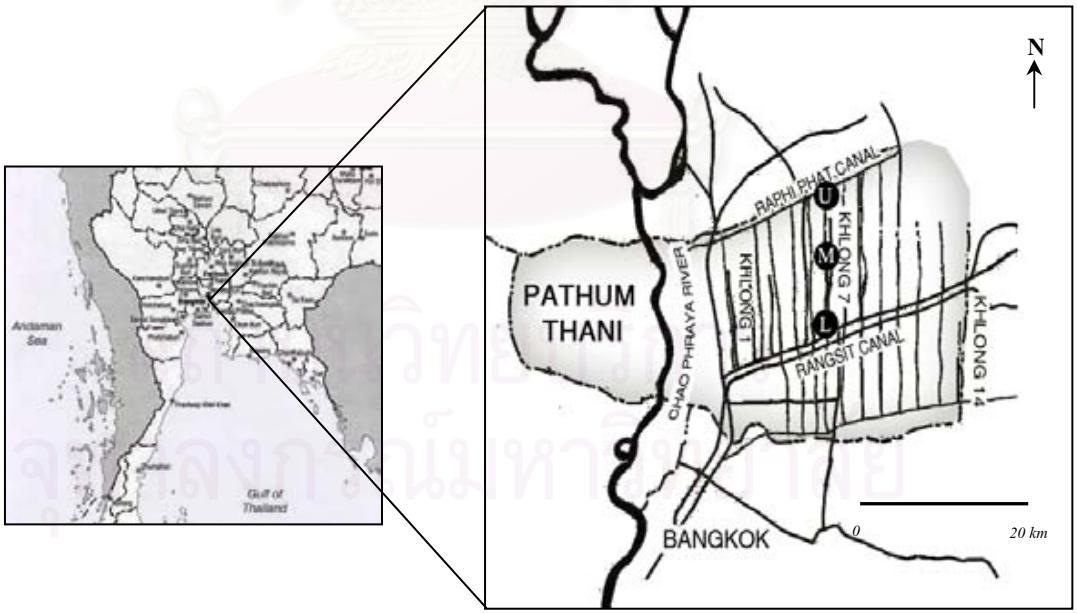


Figure 5.1 Map of Rangsit agricultural area, Pathum Thani Province, Thailand. The sampling stations are at Khlong 7; where (U) = upper stream, (M) = middle stream, and (L) = lower stream

Field samplings were conducted from June 2004 to May 2007. Triplicate samples of water, sediment, plankton, aquatic plants, invertebrates and fishes were monthly collected from the upper stream (U), middle stream (M), and lower stream (L) of Khlong 7. Water samples were contained in polyethylene bottles. Sediment and each species of organism samples were separately placed in a polyethylene bag. Plankton samples were caught using a No. 20 net with mesh opening 80 μm (APHA-AWWA-WPCF, 1980) and then put in polyethylene bottles. All samples were maintained below 4 ˚C during transportation and storage until analysis. Additionally, the physical environment data such as temperature, pH, and dissolved oxygen (DO) were monthly measured.

5.2.3 Sample extraction and clean up

5.2.3.1 Extraction of OCPRs in water

Using liquid-liquid extraction (LLE) as described in APHA (1975), the total amount of 800 mL of each surface water sample was filtered with Whatman® filter paper (i.d. 70 mm) then poured into 2-L separatory funnel. For the first LLE, the mixture of 100 mL n- hexane and dichloromethane (1:1 v/v) was added and shaken vigorously for 2 min before 2-phase separated at least 10 min. The water-phase was drained from the separatory funnel into a 1,000 mL beaker, and carefully poured the organic phase to a glass funnel containing a 20 g anhydrous sodium sulfate through a 200-mL concentrator tube. Following the second and third LLE, water-phase was poured back into the separatory funnel to re-extract with 50 mL of the same solvent mixtures. The extract was concentrated to the volume of 2 mL under a gentle stream of nitrogen using Turbo Vap® evaporator, and then analyzed with GC-μECD (5.2.4).

5.2.3.2 Extraction of OCPRs in sediment

Each sediment sample had been well-mixed and dried in a circulating air at the room temperature without sunlight exposure for 3-4 days. Dried sample was ground and sieved (500 μm) to remove stones and shells (Pridmore *et al.*, 1992). Using accelerated solvent extractor (ASE, Dionex Canada Ltd. Oakville, ON, Canada), 5 g of the sediment sample was mixed with 5 g anhydrous sodium sulfate (1:1 w/w) and placed into a 34-mL ASE-vessel, then extracted with 1:1 v/v 95% n-hexane:

dichloromethane. The sample was preheated for 5 min and extracted at 100°C with pressure 1,500 psi, for 10 min. Finally, sample was purged with nitrogen for 60 sec.

To remove sulfur contamination as previously described in Pan *et al.* (2004), the elute was cleaned up with 30-cm chromatographic column by packing 6 g of florisil layer between 2 g of activated copper powder and 10 g of anhydrous sodium sulfate layer. Three fractions of eluents were used specifically: 50 mL of 6%, 15%, and 50% of diethyl ether in petroleum ether, respectively. The elution rate was 5 mL/min by gravity. The eluates were collected in a concentrator tubes and the volume was reduced to 2 mL under a gentle stream of nitrogen for quantification with GC-μECD (5.2.4).

5.2.3.3 Extraction of OCPRs in plankton

The method was modified from DeLorenzo *et al.* (2002). The plankton mass was separated from an aliquot (30 mL) by centrifugation (2500 rpm. for 30 min). The supernatant was decanted. The plankton pellet was then washed with deionized water and recentrifuged twice as before. Afterward, plankton pellet was weighed using 4-digit balance, dissolved in 2 mL methanol and vortexed. An equal amount of hexane was then added and the contents were mixed. After phase separation, a 1-mL aliquot of hexane layer was transfer to clean up. A florisil SPE cartridge was applied for clean up using three fraction eluents: 10 mL of 6%, 15%, and 50% of diethyl ether in petroleum ether, respectively (Caleste Matos Lino and Irene Noronha da Silveira, 1997; Alvin and Lau, 2004). The elution rate was 1 mL/min by gravity. The eluates were collected in a concentrator tube and volume was reduced to 2 mL under a gentle stream of nitrogen for quantification with GC-μECD (5.2.4).

5.2.3.4 Extraction of OCPRs in aquatic plants

Using Accelerated Solvent Extraction (ASE), a mixture of 1:1 v/v 95% n-hexane:dichloromethane was used as an extracting solvent. A 5 g of blended aquatic plant was mixed with 20 g anhydrous sodium sulfate contained in the ASE-vessel. ASE condition was in the same way for prior to sediment extraction. Following the pigment removal, the same clean up technique during the plankton extraction was used and then the sample will be analyzed by GC-μECD (5.2.4)

5.2.3.5 Analysis of invertebrates

Using the standard operating procedure (SOP) for determination of chlorinated pesticides, PCB Arochlor(s) and PCB congeners in fish and biological tissue (AOAC, 2002), the whole body of each invertebrate tissue was homogenized. A 5 g of sample was mixed with 10 g anhydrous sodium sulfate in the ASE-vessel and then extracted with n-hexane:dichloromethane (1:1 v/v) using ASE (Aaron *et al*., 2003; Thongkongoum, 2005). Following the removal of fat and pigment, the same clean up technique during the plankton extraction was used and then the sample will be analyzed by GC-μECD (5.2.4).

5.2.3.6 Extraction of OCPRs in fish

A 5 g of homogenized fish was mixed with anhydrous sodium sulfate to remove water. Mixed fillet was placed into the ASE-vessel. The mixture of hexane:acetone (3:1 v/v) was used as the extracting solvent with the same operating condition as described previously in the sediment extraction (AOAC, 2002; Zhuang *et al*., 2004; Rohitrattana, 2005). The same clean up technique that was used during the plankton extraction was used and then the sample will be analyzed by GC-μECD (5.2.4).

5.2.4 Gas chromatography analysis

An Agilent 6890N GC equipped with micro Electron Capture Detector (μECD) was used for the quantification. Compound separation was completed using DB-35MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) coated with 35% diphenyl polysiloxane (J&W Scientific). Sample quantification was performed using multiple external standards. A 1.0 μL of sample was injected into the GC on splitless mode with 0.75 min vent delay. The injector and detector temperature were maintained at 260 ◦C and 300 ◦C, respectively. The oven temperature was initially maintained at 100 ºC for 2 min, and then programmed to increase at 12 ºC /min to 280 ºC and held for 10 min. Total run time was calculated to be 27.00 min. For optimum performance, the ultra-high-pure (UHP, 99.999%) helium was used as carrier gas with a flow rate at 2 mL/min linear velocity, and nitrogen (UHP) was set at 60 mL/min as make-up gas.

5.2.5 Quality control

Organochlorine pesticides (OCPs) peaks and retention times were confirmed with DB-1701 fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) coated with 14% cyanopropylphenyl and 86% diphenyl polysiloxane (J&W Scientific). A calibration curve using the external mixed standard of 17 OCPs was performed for each compound to be quantified at concentrations of 5, 10, 20, 50, and 100 ng/mL. Calibration standards were run every 10 samples and all measurements were performed in the ranges of linearity found for each compound. We considered the methods to be reliable to quantify the concentration of OCPRs in all matrices according to the AOAC Peer Verified Methods Program (1993); the limit of detection (LOD), the limit of quantitation (LOQ), the method detection limit (MDL), the relative standard deviations (RSD), and the recoveries of OCPRs in all matrices were summarized in Table 5.1.

5.2.6 Quantification of bioaccumulation, bioconcentration, and biomagnification

Bioaccumulation is the net accumulation of a contaminant in- and on- an organism from all sources in the environment (Newman, 1998). In this chapter, it refers to the ratio of concentration in organism and concentration in sediment (equation 1).

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{Concentration in aquatic organisms } (C_n)}{\text{Concentration in sediment } (C_{sediment})} \qquad (5.1)$$

Bioconcentration is the different restricted term from bioaccumulation in that the net accumulation of a contaminant in- and on- an organism is from water only (Newman, 1998). It can be estimated from equation 2.

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Concentration in aqutic organisms } (C_n)}{\text{Concentration in water } (C_{water})} \qquad (5.2)$$

Biomagnification refers to the process by which tissue concentration of bioaccumulation of contaminants increase via the food chain as they pass from one trophic level (e.g. prey) to the next (e.g. predator). Biomagnification results in

exposure to higher contaminant levels in top predators of ecosystem (Newman, 1998; NRC, 2003). It can be calculated by equation 3.

$$\text{Biomagnification factor (BMF)} = \frac{\text{The concentration from at trophic level n } (C_n)}{\text{The concentration at the next lowest trophic level } (C_{n-1})} \quad (5.3)$$

5.2.7 Food web concepts

Bioavailability processes vary greatly between predators, prey and degrader within an ecosystem. Organisms can be exposed to contaminants either from soil, sediment, and water through their diet. Invertebrates that bioconcentrate OCPs such as DDT from sediment can be eaten by other wildlife, allowing the compounds to bioaccumulate in their tissues. Eventually, an entire food chain, which refers to sequential feeding of a series of organisms, can be affected (NRC, 2003).

The susceptibility of compounds to bioconcentration, bioaccumulation, or biomagnification is a characteristic of the food web, the compound of concern, and the status of the system in terms of steady state. Biomagnificaion is generally observed for nonpolar or lipophilic contaminants that have low solubility, high log K_{ow}, and are recalcitrant in the environment and the organism. The food web concept defines interactions of interrelated food chains and takes into account species participation in multiple food chains over different trophic levels (NRC, 2003).

5.2.8 Statistical analysis

All statistical analyses were performed using SPSS software (Version 12.0). The statistical differences for mean concentration of OCPRs in water, sediment, plankton, aquatic plants, invertebrates, and fish were determined by using one-way ANOVA, followed by post hoc tests, Tamhane's T2 (equal variances not assumed).

Table 5.1 The limit of detection (LOD), the limit of quantitation (LOQ), the method detection limit (MDL), the relative standard deviations (RSD), and the recoveries of OCPRs in different matrices

Organochlorine Pesticides	LOD (ng/mL)	LOQ (ng/mL)	MDL (ppb)						
			Water (ng/mL)	*Sediment (ng/g dry wt.)*	*Plankton (ng/g wet wt.)*	*Aquatic Plants (ng/g wet wt.)*	*Fish (ng/g wet wt.)*	*Invertebrates (ng/g wet wt.)*	
								Shrimp	*Snail*
∑ HCH	0.01-0.05	0.05-0.20	0.004-0.006	1.07-1.22	0.06-0.11	1.93-3.96	0.70-1.00	0.55-2.24	0.22-2.37
Heptachlor & Heptachlor epoxide	0.001-0.02	0.002-0.07	0.001-0.003	1.11-0.67	0.15-0.23	1.94-2.37	1.09-1.40	1.46-2.24	1.80-3.89
DDT & derivatives	0.002-0.04	0.01-0.10	0.002-0.003	0.66-0.74	0.05-0.19	1.93-2.34	1.06-1.40	1.46-3.32	0.64-3.89
∑ Endosulfan	0.002-0.003	0.007-0.009	0.003-0.01	0.75-1.38	0.02-0.20	1.78-2.32	0.77-1.20	1.57-3.82	1.06-5.17

Organochlorine Pesticides	RSD (%)						
	Water	*Sediment*	*Plankton*	*Aquatic Plants*	*Fish*	*Invertebrates*	
						Shrimp	*Snail*
∑ HCH	8.41-11.07	3.24-3.76	0.49-0.87	7.21-12.21	2.75-5.38	1.74-7.66	0.72-8.08
Heptachlor & Heptachlor epoxide	6.02-8.76	1.89-3.34	1.40-1.79	3.65-5.94	4.86-10.16	5.80-7.15	0.53-5.97
DDT & derivatives	4.22-7.64	1.87-3.69	0.47-1.67	5.06-8.60	4.99-7.59	3.74-7.52	1.42-8.38
∑ Endosulfan	7.99-11.86	2.24-2.99	0.20-2.15	5.64-7.31	3.17-5.98	4.80-9.87	2.13-12.78

Organochlorine Pesticides	Matrices Spiked Recovery (%)						
	Water	*Sediment*	*Plankton*	*Aquatic Plants*	*Fish*	*Invertebrates*	
						Shrimp	*Snail*
∑ HCH	95-120	75-78	87-101	74-79	96-128	68-104	69-85
Heptachlor & Heptachlor epoxide	87-114	83-84	84-100	74-75	112-115	83-92	70-71
DDT & derivatives	77-116	86-91	90-103	71-103	82-109	88-103	78-106
∑ Endosulfan	96-117	89-90	89-100	79-83	85-125	77-109	82-117

5.3 Results and Discussions

5.3.1 The physical environment of Khlong 7

The physical parameters of surface water in Khlong 7 Rangsit agricultural area were collected at the same time of biotic sample collection from June 2004 to May 2007. The mean surface temperature throughout the study period was 30.9 ± 2.1 ◦C. The mean dissolved oxygen was 4.9 ± 1.3 mg/l which was existing in the range of class 2 (6 mg/L) and class 3 (4 mg/L) of surface water classifications (The Ministry of Science Technology and Energy, 1986). Furthermore, the pH was found existing in the range of 5.0-7.5 in which the standard range of the pH in class 2, 3, and 4 were recommended between the range of 5.0-9.0 (The Ministry of Science Technology and Energy, 1986).

5.3.2 Organochlorine pesticides in water, sediment, plankton, aquatic plants, invertebrates, and fish

Table 5.2 illustrates the concentration means with standard error (S.E.) values of OCPRs in water (ng/mL), sediment (ng/g dry wt.), plankton (ng/g wet wt.), aquatic plants (ng/g wet wt.), invertebrates (ng/g wet wt.), and fishes (ng/g wet wt.) from Khlong 7 Rangsit agricultural area from June 2004 to May 2007. All selected OCPRs including of ∑ hexachlorocyclohexane (α-, γ-, β- and δ-HCH), heptachlor & heptachlor epoxide, DDT & derivatives, and ∑ endosulfan (α-, β- and -sulfate) were found. The concentration of OCPRs in water was compared with the Criteria Maximum Concentration (CMC) and the Criterion Continuous Concentration (CCC) reported in the National Recommended Water Quality Criteria (US EPA, 2006). All OCPRs values did not exceed the CMC recommendation limits. All OCPRs mean values in water were in the range of those samples collected from other agricultural areas in Thailand on 1997 (Anat and Paul, 2000). ∑ endosulfan (0.08 ng/mL) was the highest concentration found in water probably because endosulfan has been illegally used to control the apple snails and various pests in paddy fields. Furthermore, ∑ HCH, DDT and derivatives, and heptachlor & heptachlor epoxide were found because they were banned recently (Thirakhupt *et al.*, 2006a).

In the sediment, the concentrations of heptachlor & heptachlor epoxide (14.67 ng/g dry wt.) and DDT and derivatives (12.05 ng/g dry wt.) were high because the octanol-water partitioning coefficients (K_{ow}) of heptachlor & heptachlor epoxide (log K_{ow}= 4.4-5.5) and DDT and derivatives (log K_{ow}=5.5-6.19) are greater than HCH (log K_{ow}= 3.72-4.14) and endosulfan (log K_{ow}= 3.55-3.62). As a result, those compounds were better trapped by sediment particles than dissolved in water. However, DDT and derivatives residue found in sediment from Victoria harbor, Hong Kong was 10.2 ng/g dry wt. (Hong *et al*., 1999) and its concentration in sediment of Macao estuary, China was 12.8 ng/g dry wt. (Zhang *et al*., 1999), as much as shown in Table 5.2 (12.05 ng/g dry wt.). In addition, the residue of ∑ HCH from the northern coast, Vietnam (Nhan *et al*., 1999) reported at 8.53 ng/g dry wt. in sediment related to the result of ∑ HCH (9.36 ng/g dry wt.) in Table 5.2.

For the aquatic plant, *Eichhornia crassipes*, the concentrations of OCPRs were ∑ HCH (5.44 ng/g wet wt.) < heptachlor & heptachlor epoxide (5.90 ng/g wet wt.) < ∑ endosulfan (7.91 ng/g wet wt.) < DDT and derivatives (9.25 ng/g wet wt.).

The concentration of OCPRs in plankton showed that DDT and derivatives (3.65 ng/g wet wt.) > ∑ endosulfan (3.29 ng/g wet wt.) >∑HCH (1.80 ng/g wet wt.) > heptachlor & heptachlor epoxide (1.79 ng/g wet wt.).

In case of the invertebrates, the residues of DDT and derivatives in *Macrobrachium lanchesteri* (53.04 ng/g wet wt.), *Pomacea sp.* (47.83 ng/g wet wt.), and *Filopaludina mertensi* (79.62 ng/g wet wt.) were higher than other OCPRs. Both *Macrobrachium lanchesteri* and *Pomacea sp.*, the concentrations of OCPRs were heptachlor & heptachlor epoxide < ∑ HCH < ∑ endosulfan < DDT and derivatives. However, the concentrations of OCPRs in *Filopaludina mertensi* were heptachlor & heptachlor epoxide < ∑ endosulfan < ∑ HCH < DDT and derivatives.

Among 10 fish species, *Channa striatus* , the top predator, was found to have the highest residue of all OCPRs including ∑ HCH (20.95 ng/g wet wt.), heptachlor & heptachlor epoxide (28.64 ng/g wet wt.), DDT and derivatives (57.66 ng/g wet wt.), and ∑ endosulfan. (46.22 ng/g wet wt.). The amount of DDT and derivatives residues in *Channa striatus* were similar to those reported by Kumblad *et al*. (2001) who in *Channa striatus* collected from Songkhla lake, Thailand which were in the range of

33-87 ng/g. Furthermore, Therdteppitak and Yammeng (2002) reported that the contaminations of ∑ HCH found in *Channa striatus* and *Puntius gonionotus* collected from commercial fish at Saparnpra, Bangkok and Bang Bau, Samutprakarn Province, Thailand were 35.0 and 14.2 ng/g wet wt., respectively. Additionally, the contamination of DDT and derivatives and ∑ endosulfan in *Oreochromis niloticus* collected from Lake Victoria, Tanzanian were reported at 0.03 and 0.2 mg/kg fresh weight, respectively (Henry and Kishimba, 2006). Both of those residues were greater than the result shown in Table 5.2 (DDT and derivatives 15.41 ng/g wet wt. and ∑ endosulfan 17.71 ng/g wet wt.). Moreover, the residue of heptachlor & heptachlor epoxide and DDT and derivatives in *Puntius gonionotus, Oreochromis niloticus, Oxyeleotris marmoratus,* and *Macrognathus siamensis* shown in Table 5.2 were greater than the residue of OCPRs from fish collected from 3 reserviors in the northern of Thailand such as Bueng Boraphed reservoir, Nonghan, and Kwanpayao which were 0.006, 0.002, 0.002, and 0.002 mg/kg wet wt., respectively for heptachlor & heptachlor epoxide and were 0.008, 0.002, 0.018, and 0.009 mg/kg wet wt., respectively for DDT and derivatives. Additionally, DDT and derivatives in *Oreochromis niloticus* from Kenyan lakes were 0.009 mg/kg wet wt. (Wandiga, 2001) in which the residue was less than those shown in Table 5.2.

Figure 5.2 illustrated the mean concentration of OCPRs in different environmental compartments from Khlong 7, Rangsit agricultural area. The pattern of increasing distribution of heptachlor & heptachlor epoxide, DDT and derivatives, and ∑ endosulfan residues in organisms were significantly different (ANOVA, $p \leq 0.05$) following: plankton < aquatic plant < vertebrates < invertebrates, respectively, except for the residue of ∑ HCH in aquatic plant and vertebrates, which were not significantly different (Tamhane's T2, $p \geq 0.05$). However, the distribution patterns of OCPRs in the water were statistically lower than these in the sediment (Tamhane's T2, $p \leq 0.05$).

Table 5.2 The mean concentration of organochlorine pesticide residues in water (ng/mL), sediment (ng/g dry wt.), aquatic plants (ng/g wet wt.), plankton (phyto- and zoo- plankton, ng/g wet wt.) invertebrates (ng/g wet wt.), and fish (ng/g wet wt.) from Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand from June 2004 to May 2007

Environment Compartments	Foraging behaviors	n	The Concentration of OCPRs (mean ± S.E.) (ppb)			
			∑ HCH	Heptachlor & heptachlor epoxide	DDT & derivatives	∑ Endosulfan
Water[*]	-	108	0.01±0.002	0.007±0.001	0.02±0.001	0.08±0.01
Sediment[♣]	-	108	9.36±0.27	14.67±0.48	12.05±0.30	6.36±0.25
Aquatic plants[†]**:**						
Eichhornia crassipes (water hyacinth)	producer	84	5.44±0.32	5.90±0.21	9.25±0.55	7.91±0.49
Plankton[†] phyto- & zoo- plankton	producer	51	1.80±0.34	1.79±0.47	3.65±0.58	3.29±0.28
Invertebrates[†]**:**						
Macrobrachium lanchesteri (lanchester's freshwater prawn)	detritivore	93	27.08±2.12	14.52±0.85	53.04±7.85	36.69±5.71
Filopaludina mertensi (pond snail)	detritivore	57	42.27±4.59	18.92±2.30	79.62±8.81	27.87±3.44
Pomacea sp. (apple snail)	detritivore	72	34.34±2.64	19.02±1.54	47.83±5.06	36.51±3.97
Vertebrates (fish)[†]**:**						
Trichogaster microlepis (moonbeam gourami)	herbivore	24	4.08±0.50	4.32±0.52	23.75±2.92	7.80±0.79
Oreochromis niloticus (Nile tilapia)	omnivore	3	8.37±0.43	6.16±0.07	15.41±0.11	17.71±0.13
Puntius gonionotus (silver barb)	omnivore	30	2.13±0.38	3.35±0.39	4.16±0.61	3.18±0.49
Trichogaster trichopterus (three-spot gourami)	omnivore	24	3.71±0.56	4.88±0.43	12.66±1.41	11.90±1.62
Anabas testudineus (climbing perch)	carnivore	6	2.13±0.53	3.52±1.26	5.71±1.40	10.64±4.21
Channa striatus (snakehead)	carnivore	9	20.95±0.51	28.64±0.50	57.66±1.08	46.22±0.67
Macrognathus siamensis (spiny eel)	carnivore	9	5.09±0.53	4.46±0.73	44.76±8.06	61.23±1.36
Oxyeleotris marmoratus (marbled sleeper)	carnivore	15	9.87±1.17	21.32±2.74	25.71±2.85	32.72±3.82
Parambassis siamensis (glassfish)	carnivore	15	11.86±0.14	27.91±0.64	23.73±0.34	40.02±0.52
Pristolepis fasciatus (catopra)	carnivore	3	5.85±0.11	8.96±0.06	14.39±0.12	33.19±0.08

[*] ng/mL, [♣] ng/g dry wt., and [†] ng/g wet wt

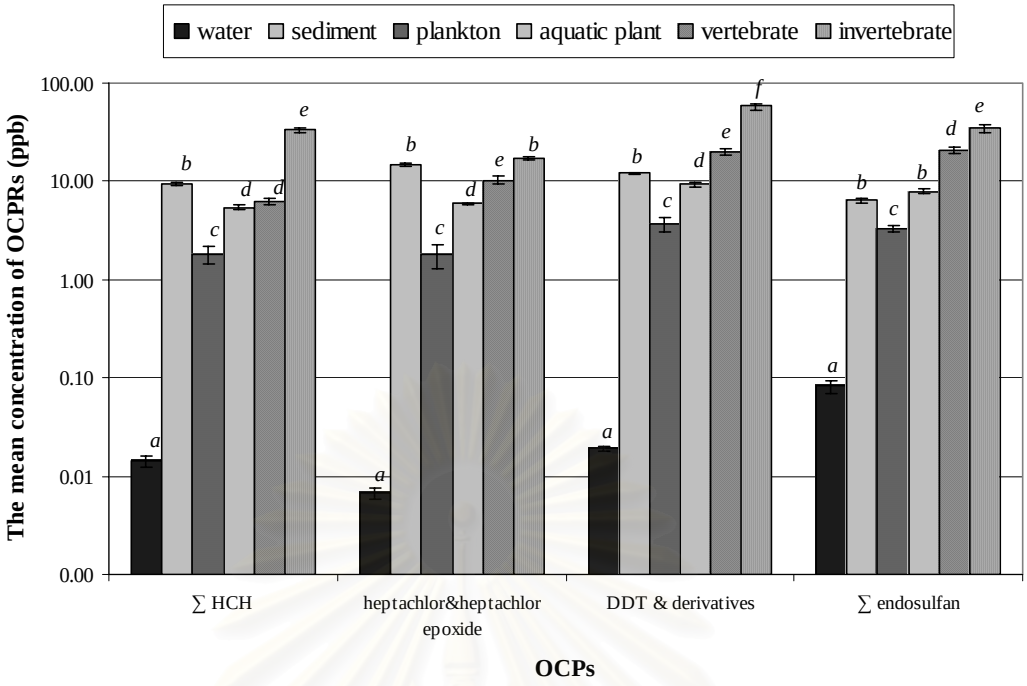


Figure 5.2 The mean concentration of organochlorine pesticide residues in different environmental compartments from Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand (For each OCPs, the different letters on the top of bar chart are significantly different at $p \leq 0.05$)

Figure 5.3 indicated that the mean concentrations of each OCP in Khlong 7, Rangsit agricultural area were significantly different among the plankton, producer, herbivore, omnivore, carnivore, and detritivore (ANOVA, $p \leq 0.05$). The result of multiple means comparison, Tamhane's T2, for each OCP was presented by the italic letters on the top of bar chart. Most concentrations of ∑ HCH, heptachlor & heptachlor epoxide, and ∑ endosulfan in both carnivore and ditritivore were significantly higher than that in plankton, producer, herbivore, and omnivore (Tamhane's T2, $p \leq 0.05$), but only DDT and derivatives had no difference between herbivore and carnivore (Tamhane's T2, $p > 0.05$). The high accumulation of OCPs in carnivore may be because of its high position in the food chain (Borgå *et al.*, 2001) and in ditritivore may be explained by its foraging behavior and niche which occurred at the bottom of the canal as benthic fauna. Nevertheless, Pérez-Ruzafa *et al.* (2000) indicated that bioaccumulation depended not only on the feeding behavior of animal species, but also on a number of different factors such as ages, sexes, and stages in the annual breeding cycle.

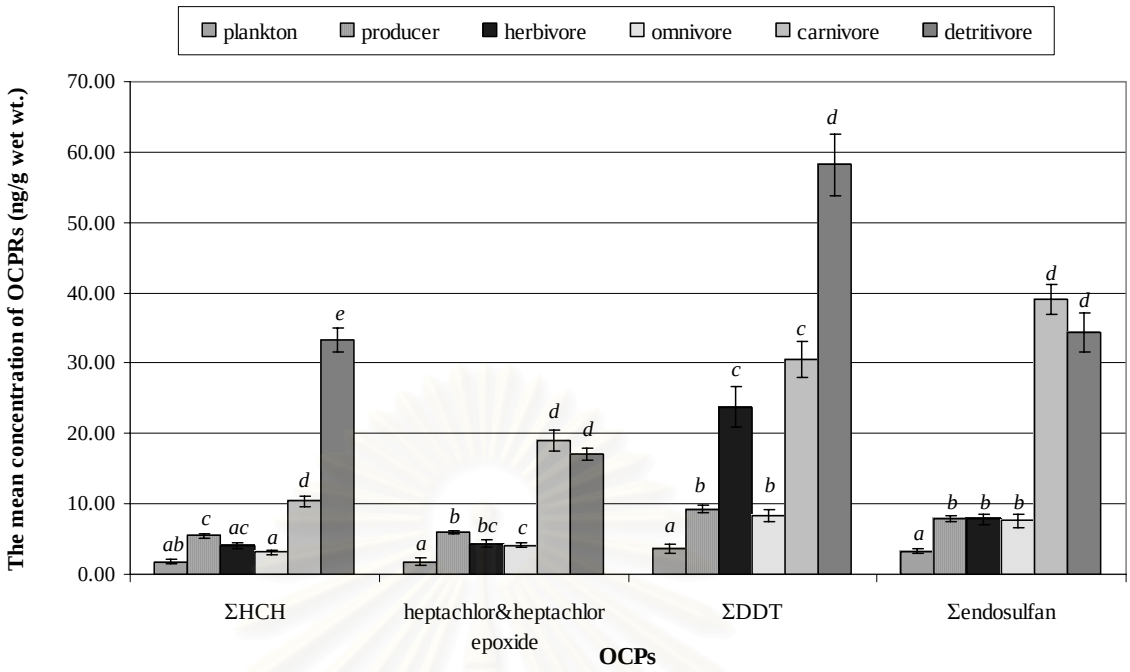


Figure 5.3 The mean concentration of organochlorine pesticide residues in plankton, producer, herbivore, omnivore, carnivore, and detritivore in Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand (For each OCPs, the different letters on the top of bar chart are significantly different at $p \leq 0.05$)

5.3.3 Bioaccumulation, bioconcentration, and biomagnification in the aquatic food web

Figure 5.4, 5.5, 5.6, and 5.7 showed the bioaccumulation factor (BAF), bioconcentration factor (BCF), and biomagnification factor (BMF) of ∑ HCH, heptachlor & heptachlor epoxide, DDT and derivatives, and ∑ endosulfan, respectively in the aquatic food web of Khlong 7, Rangsit agricultural area. Invertebrate species, primary consumers, such as *Macrobrachium lanchesteri*, *Pomacea* sp., and *Filopaludina mertensi* showed the BAF in the range of 2.9-4.5 for ∑ HCH, 1.0-1.3 for heptachlor & heptachlor epoxide, 4.0-4.6 for DDT and derivatives, and 4.4-5.8 for ∑ endosulfan. *Macrobrachium lanchesteri* and *Pomacea* sp. showed the maximum BAF of ∑ endosulfan which were 5.8 and 5.7, repectively. The highest BAF of DDT and derivatives was found in *Filopaludina mertensi* (6.6).

In the case of BCF, the maximum BCF in *Eichhornia crassipes*, macrophyton as a producer, was presented by heptachlor & heptachlor epoxide (842.9) followed by ∑ HCH (554.0), DDT and derivatives (462.5), and ∑ endosulfan (98.9), respectively.

The BCF in this chapter are compared with the values found in other species of aquatic plant such as BCF of γ-HCH (lindane), one of 4 isomers of HCH, was 38.15 in *Hydrilla verticillata* (Mark and Klaine, 1992). The BCF of ∑ HCH, ∑ endosulfan, and DDT and derivatives in *Chaetomorpha linum* were 1,081, 37, and 10,460, respectively and reaching value of 30,980 times the concentration in water of heptachlor & heptachlor epoxide in the *gammaridae* which was greater than in this chapter (Pérez-Ruzafa *et al*, 2001).

Besides the BCF in plankton were 255.7, 182.5, 180.0, and 41.1 in heptachlor & heptachlor epoxide, DDT and derivatives, ∑ HCH, and ∑ endosulfan, respectively. For plankton-eating invertebrate such as *Macrobrachium lanchesteri*, the BMF (*Macrobrachium lanchesteri*/plankton) for ∑ HCH equals 15.0 which were more than in heptachlor & heptachlor epoxide (8.1), DDT and derivatives (14.5), and ∑ endosulfan (11.2).

In the case of BMF of plankton-eating fish, there were *Puntius gonionotus, Parambassis siamensis, Trichogaster trichopterus,* and *Trichogaster microlepis.* The BMF (*Parambassis siamensis*/plankton) for∑ HCH (6.6), heptachlor & heptachlor epoxide (15.6), and ∑ endosulfan (18.6) were higher when compared with other fish in this dissertation. The lowest BMF of plankton-eating fish for heptachlor & heptachlor epoxide, ∑ HCH, DDT and derivatives, and ∑ endosulfan was found in *Puntius gonionotus* which equal to 1.9, 1.2, 1.1, and 1.0, respectively.

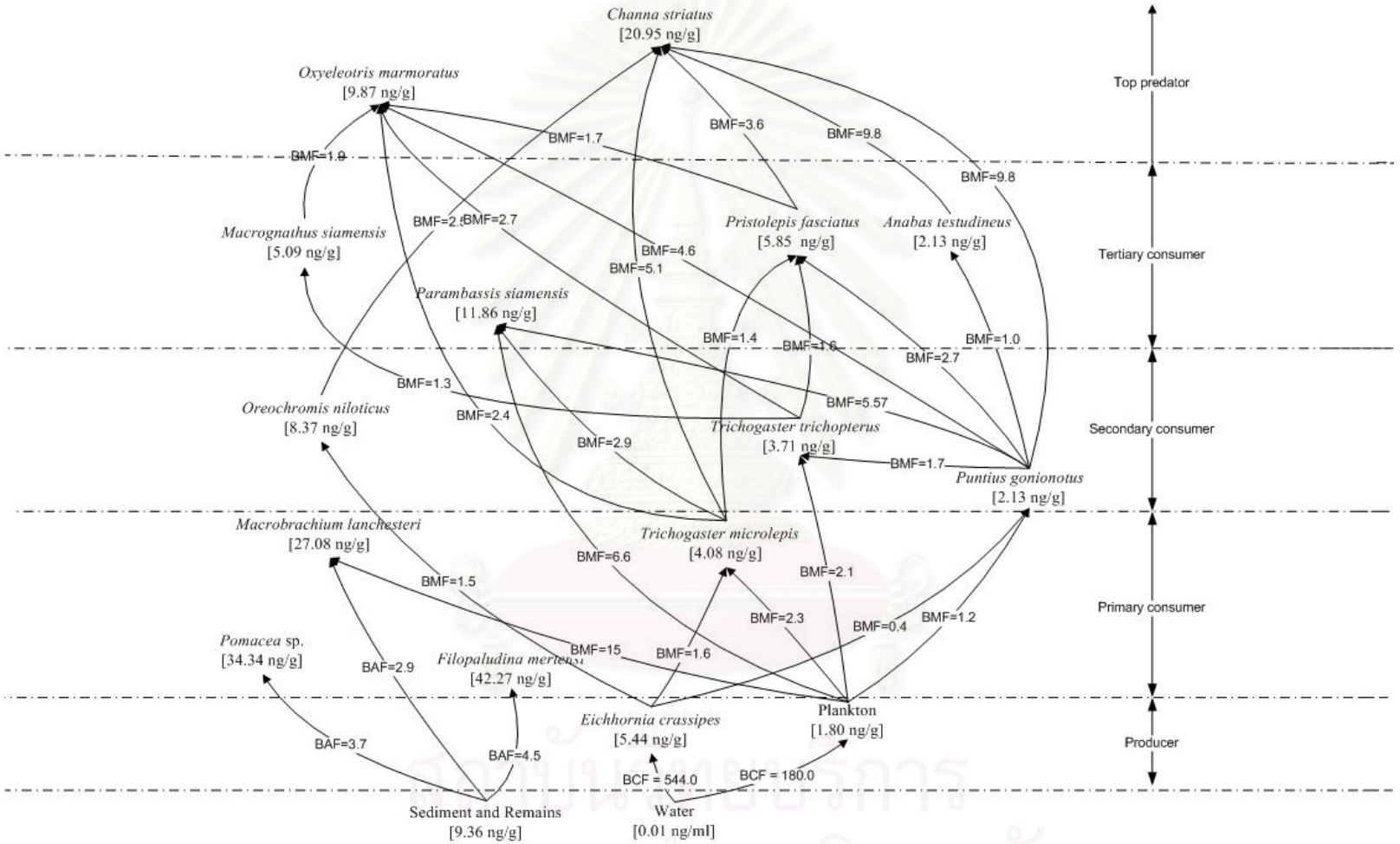
Previous studies have shown that BCF of different organochlorines in fish were related to the octanol–water partitioning coefficient (K_{ow}) (Ritter, 1995). Because of high log K_{ow} of DDT and derivatives (5.5-6.19), heptachlor & heptachlor epoxide (4.4-5.5), and ∑ HCH (3.72-4.14), Ritter (1995) reported that the BCF in *Pimephales promelas* (fathead minnows) exposed to DDT and derivatives at 2.0 µg/L for 14 days was 69,100. Furthermore, *Pimephales promelas* exposed to ∑ HCH at 4.8 µg/L for 32 days was 22,000; heptachlor & heptachlor epoxide was 14,400. With a BCF of 2,755 (Hansen, 1993), ∑ endosulfan can be considered as having a moderate potential for bioaccumulation (log K_{ow}=3.55-3.62). As we have calculated BCF, the top predator, mainly the *Channa striatus* (snakhead fish) showed the BCF of heptachlor & heptachlor epoxide, ∑ HCH, DDT and derivatives, and ∑ endosulfan at 4,091.4, 2,883.0, 2095.0, and 458.6, respectively. The BCF of these compounds were

lower than reported in Ritter (1995), it may be because the natural water comtaminated with OCPs from Khlong 7 were detected in small amounts (0.007-0.08 ng/mL). Howerver, both trends of BCFs are similar in that the more log K_{ow} is high in value, the more BCF is also high in organism.

For BMF in fish, the uptake of contaminants generally takes place from water across respiratory surfaces and from ingested food (Borgå *et al*, 2001). In Figure 5.4, 5.5, 5.6, and 5.7, most BMFs of ∑ HCH, heptachlor & heptachlor epoxide, DDT and derivatives, and ∑ endosulfan presented the increasing of BMF (BMF > 1.0) through the food web including interspecific relationship and intraspecific relationship trophic level due to rapid and high efficient energy transfer coupled with lipid content in predators (Norstrom *et al.*, 1988). Remarkably in broad perspective, the more prey species along food chain were uptaken, the lower BMF value between fish and their prey were presented such as BMF (*Channa striatus/Pristolepis fasciatus*) and BMF (*Pristolepis fasciatus/ Puntius gonionotus*) for DDT and derivatives were 4.0 and 3.5, respectively while BMF (*Channa striatus/Puntius gonionotus*) for DDT and derivatives was 13.9. Likewise, ∑ HCH and heptachlor & heptachlor epoxide showed the same BMF behavior. On the other hand, the contaminants may be eliminated through metabolism and excretion resulting in biomagnification reduction (Borgå *et al*., 2001) as same as BMFs (*Puntius gonionotus*/*Eichhornia crassipes*) for all OCPs were less than 1.0, this phenomenon is called trophic depletion or trophic dilution (Newman, 1998).

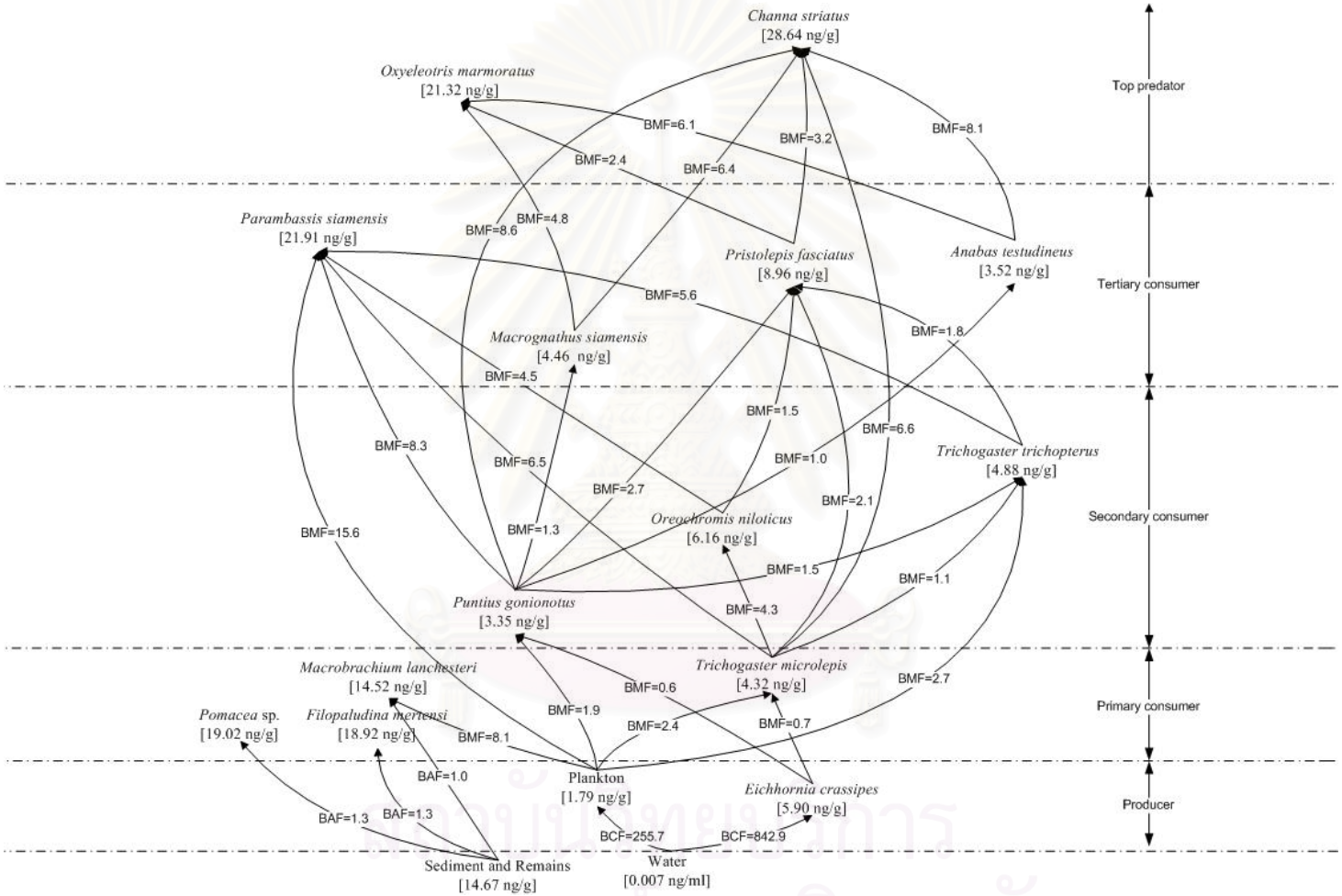
5.4 Conclusions

This study provides the environmental data for a better understanding of the fate of ∑ HCH, heptachlor & heptachlor epoxide, DDT and derivatives, and ∑ endosulfan in tropical aquatic ecosystem. Although these OCPs were banned, their residues are still circulated and magnified through the food chain from the lowest up to the highest trophic level. It also reveals that further studies on human health risk assessment for susceptible people of Rangsit agricultural communities are required.



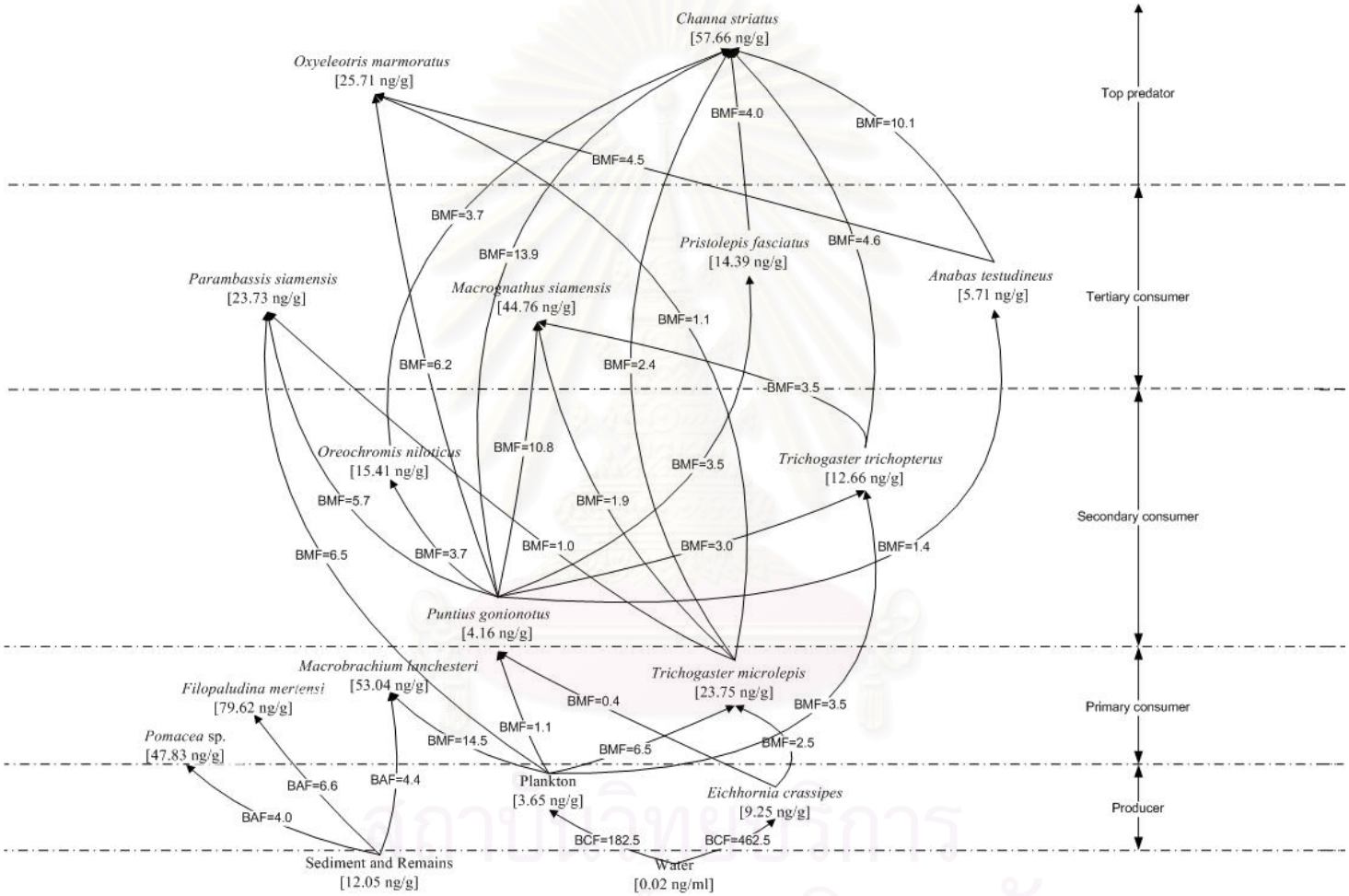
** BAF, BCF, and BMF are based on whole body (WB) of organism measurements and calculated on a wet weight basis.*

Figure 5.4 The bioaccumulation, bioconcentration, and biomagnification of ∑ hexachlorocyclohexane (HCH) in the aquatic food web of Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand



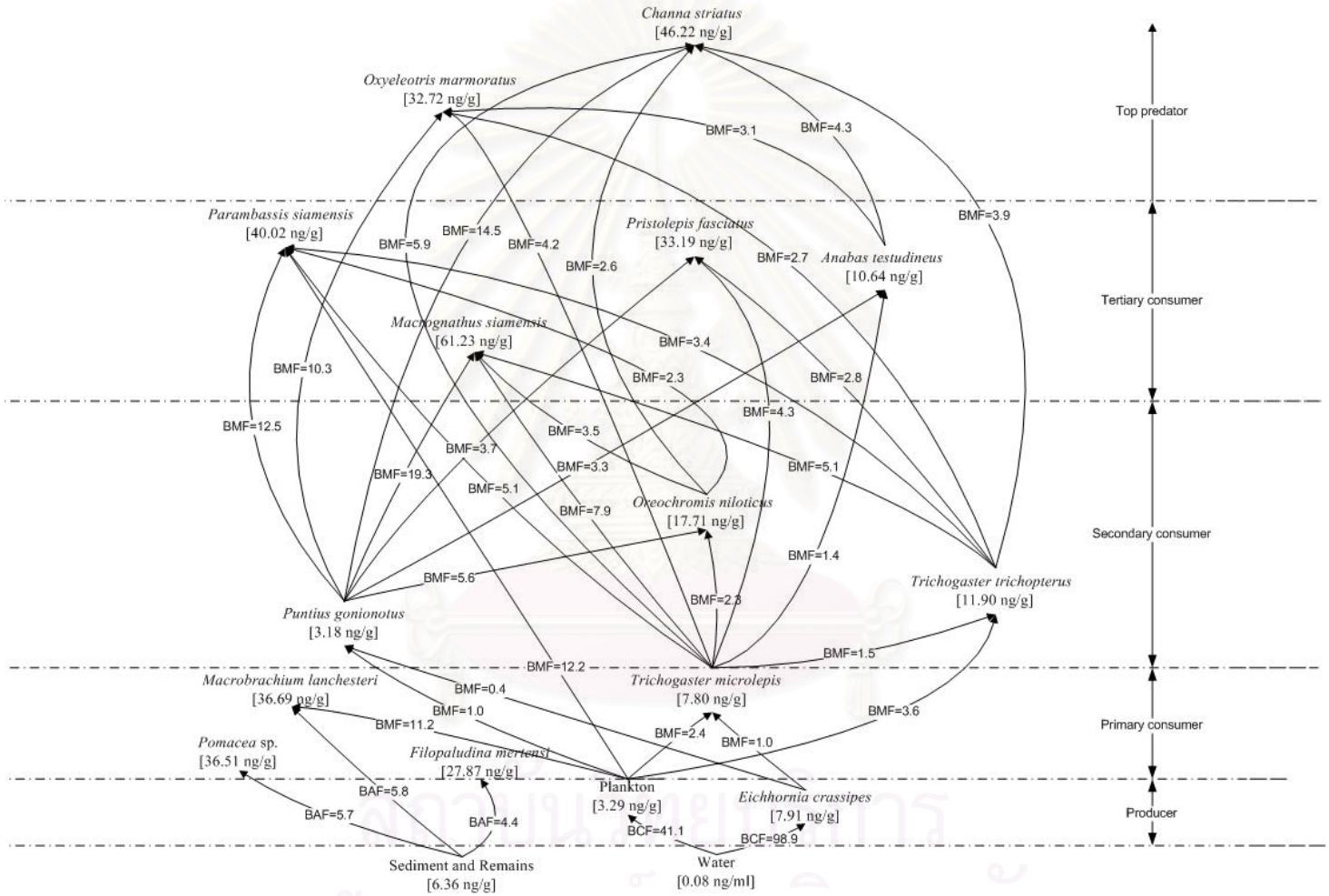
** BAF, BCF, and BMF are based on whole body (WB) of organism measurements and calculated on a wet weight basis.*

Figure 5.5 The bioaccumulation, bioconcentration, and biomagnification of heptachlor & heptachlor epoxide in the aquatic food web of Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand



** BAF, BCF, and BMF are based on whole body (WB) of organism measurements and calculated on a wet weight basis.*

Figure 5.6 The bioaccumulation, bioconcentration, and biomagnification of DDT & derivatives in the aquatic food web of Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand



** BAF, BCF, and BMF are based on whole body (WB) of organism measurements and calculated on a wet weight basis.*

Figure 5.7 The bioaccumulation, bioconcentration, and biomagnification of ∑ Endosulfan in the aquatic food web of Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand

CHAPTER VI

A PRELIMINARY HUMAN HEALTH RISK ASSESSMENT OF ORGANOCHLORINE PESTICIDE RESIDUES ASSOCIATED WITH AQUATIC ORGANISMS FROM RANGSIT AGRICULTURAL AREA, CENTRAL THAILAND

6.1 Introduction

In Thailand, the use of organochlorine pesticides (OCPs), such as dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), aldrin, dieldrin, heptachlor, etc. started in the 1950s and reached maximum use in the 1970s through the 1990s. Although most OCPs have been banned for nearly two decades, residue is still found in the majority of aquatic ecosystem compartments, which includes water, sediment, aquatic organisms, and aquatic plants. This is especially prominent in the Rangsit agricultural area, in the central part of Thailand (Rohitrattana, 2005; Thongkongoum, 2005; Thirakhupt *et al.*, 2006a; Siriwong *et al.*, 2007). These pesticide contaminants biologically accumulate at high concentration levels in the tissue of aquatic organisms and increase at each successive level of the food chain. Even extremely low concentrations of bioaccumulative pollutants are detected in water or bottom sediments. Pollutants tend to accumulate in the fat tissue of aquatic organisms or they selectively bind to muscle tissues in fish. Concentrations may be high enough to pose health risks to consumers (US EPA, 2000a).

Human health risk assessments have been underway worldwide to examine the effects of exposure to toxic contaminants in various environmental media and foodstuff. Toxic chemicals accumulate in fish, shellfish, and plants that exist in contaminated water and by consuming these organisms humans are exposed to these toxic chemicals (NRC, 1993). Food consumption databases have been established to provide the necessary information for assessing the health risks associated with consumption of contaminated food in countries, such as the U.S. There has been a tendency for risk assessors in countries that do not have comprehensive food

consumption databases to adopt the American food consumption data for risk assessment (NRC, 1993; Dougherty *et al.*, 2000).

In this study, we aimed to estimate the health risk of local populations who consumed edible aquatic organisms from Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand. Additionally, to provide a more accurate assessment of the risks, it is necessary to establish a specific aquatic organism consumption database for the local populations. To do this a questionnaire survey will be used instead of using the American food consumption data for risk assessments as reported in NRC (1993) and Dougherty *et al.* (2000).

6.2 Materials and Methods

6.2.1 Pesticide standard and chemicals

Seventeen organochlorine pesticide standards for α-, γ-, β- and δ-HCH, heptachlor, heptachlor epoxide, aldrin, α-endosulfan, β-endosulfan, endosulfan sulfate 4,4'-DDE, 4,4'-DDD, 4,4'-DDT, dieldrin, endrin, endrin aldehyde, and methoxychlor were obtained from Supelco (Bellefonte, PA, USA). A stock of the standard mixture containing 17 pesticides was prepared in 99% n-hexane at a concentration of 1,000 ng/mL and stored at -4 ◦C in a refrigerator. Working standard solutions were prepared at the concentration of 0.001–100 ng/mL by volume and then diluted with 99% n-hexane.

Residue analysis solvents such as 95% and 99% n-hexane, dichloromethane, diethyl ether, and petroleum ether were pesticide grade solvents purchased from Labscan Asia Co. Ltd. All chemical reagents were purchased from Fluka Riedel-de Haën i.e. florisil (60-110 mesh) and anhydrous sodium sulfate (granular) which was heated overnight at 300 ◦C. The 500 mg florisil SPE cartidges were purchased from Alltech Associates Inc.

All Pyrex® glassware was well-cleaned with laboratory detergent purchased from EMC-IMEX co., Ltd., then sequentially rinsed with distilled water and acetone. Finally, washed glassware was baked in an oven at 300 ◦C overnight.

6.2.2 Study area and sampling

Rangsit agricultural area is located at the central part of Thailand in Pathum Thani Province. This agricultural area has a man-made irrigation-network-system consisting of 14 sub-canals (Khlong). These sub-canals are divided by Rangsit-Prayulasakdi canal into an upper and lower part. The study area is situated at Khlong 7, a 20-km sub-canal, on the upper part of the irrigation-network-system. Khlong 7 links Raphi Phat canal at the upstream side (14°12′38.00″N, 100°45′18.38″E) and Rangsit-Prayulasakdi canal at the downstream side (14°01′51.25″N, 100°45′21.25″E) (Figure 6.1).

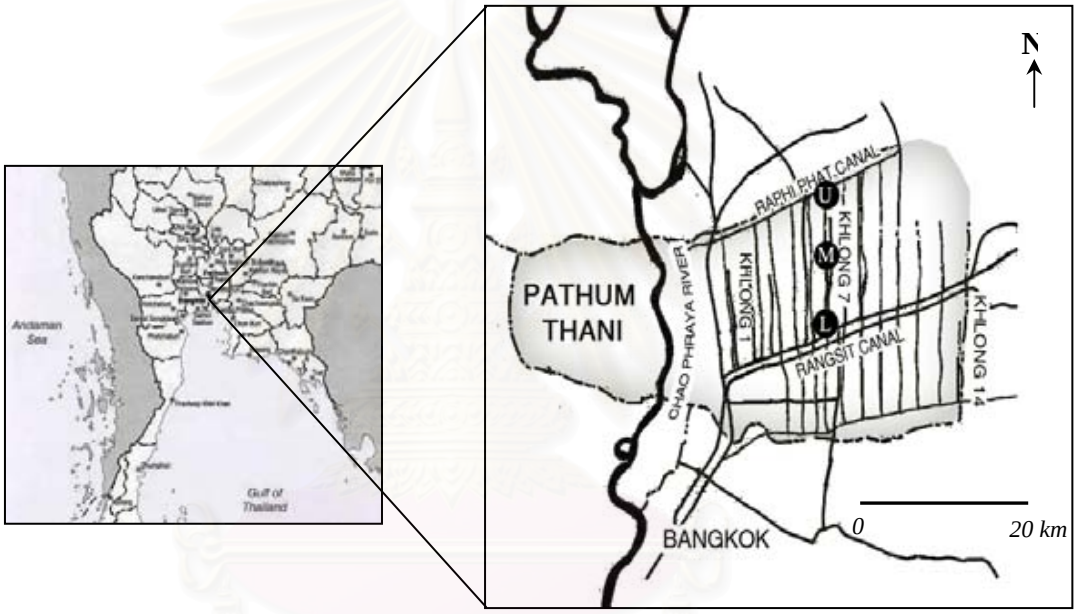


Figure 6.1 Map of Rangsit agricultural area, Pathum Thani Province, Thailand. The sampling stations are at Khlong 7; where (U) = upper stream, (M) = middle stream, and (L) = lower stream

Field samplings were conducted from June 2004 to May 2007. Triplicate samples of vegetables, invertebrates and fishes were collected monthly from the upper stream (U), middle stream (M), and lower stream (L) of Khlong 7 and maintained below 4 °C during transportation and storage until analysis.

6.2.3 Sample extraction and clean up

6.2.3.1 Extraction of OCPRs in vegetables

Using Accelerated Solvent Extraction (ASE), a mixture of 1:1 v/v 95% n-hexane:dichloromethane was used as an extracting solvent. A 5 g of blended vegetable was mixed with 20 g anhydrous sodium sulfate contained in the ASE-vessel. ASE conditions called for preheating for 5 min and extracting at 100°C with a pressure of 1,500 psi, for 10 min. Finally, the sample was purged with nitrogen for 60 sec. Following the pigment removal (Caleste Matos Lino and Irene Noronha da Silveira, 1997; Alvin and Lau, 2004), a SPE-florisil cartridge was applied for clean up using three fraction eluents: 10 mL of 6%, 15%, and 50% of diethyl ether in petroleum ether, respectively. The elution rate was 1 mL/min by gravity. The eluates were collected in a concentrator tube and the volume was reduced to 2 mL under a gentle stream of nitrogen for quantification with GC-μECD (6.2.4).

6.2.3.2 Extraction of OCPRs in invertebrates

Using the standard operating procedure (SOP) for determination of chlorinated pesticides, PCB Arochlor(s) and PCB congeners in fish and biological tissue (AOAC, 2002), the whole body of each invertebrate tissue was homogeneized. Five grams of sample was mixed with 10 g anhydrous sodium sulfate in the ASE-vessels and then extracted with n-hexane:dichloromethane (1:1 v/v) by using the operating ASE condition as same as with vegetables (Aaron et al., 2003; Thongkongoum, 2005). Following removal of fat and pigment, the same clean up technique during the vegetable extraction was used and then the sample will be analyzed by GC-μECD (6.2.4).

6.2.3.3 Extraction of OCPRs in OCPRs in fish

A 5 g of homogenized fish was mixed with anhydrous sodium sulfate to remove water. Mixed fillet was placed into the ASE-vessel. The mixture of hexane:acetone (3:1 v/v) was used as the extracting solvent with the same operating condition as described previously in the vegetable extraction (AOAC, 2002; Zhuang *et al*., 2004; Rohitrattana, 2005). The same clean up technique that was used during

the vegetable extraction was used and then the sample will be analyzed by GC-μECD (6.2.4).

6.2.4 Gas chromatography analysis

An Agilent 6890N GC equipped with micro Electron Capture Detector (μECD) was used for the quantification. Compound separation was completed using DB-35MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) coated with 35% diphenyl polysiloxane (J&W Scientific). Sample quantification was performed using multiple external standards. A 1 μL of sample was injected into the GC on splitless mode with 0.75 min vent delay. The injector and detector temperature were maintained at 260 ˚C and 300 ˚C, respectively. The oven temperature was initially maintained at 100 ºC for 2 min, and then programmed to increase at 12 ºC /min to 280 ºC and held for 10 min. Total run time was calculated to be 27.00 min. For optimum performance, the ultra-high-pure (UHP, 99.999%) helium was used as carrier gas with a flow rate at 2 mL/min linear velocity, and nitrogen (UHP) was set at 60 mL/min as make-up gas.

6.2.5 Quality control

Organochlorine pesticides (OCPs) peaks and retention times were confirmed with DB-1701 fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) coated with 14% cyanopropylphenyl and 86% diphenyl polysiloxane (J&W Scientific). A calibration curve using the external mixed standard of 17 OCPs was performed for each compound to be quantified at concentrations of 5, 10, 20, 50, and 100 ng/mL. A calibration standards were run every 10 samples and all measurements were performed in the ranges of linearity found for each compound. In Appendix A, validation data showed essentially quantitative recovery in the range of 82-125, 68-115, 69-117, and 66-116 %, excellent precision in the range of 2.75-10.16, 1.74-9.87, 0.72-12.78, and 3.15-12.21 %RSD, method detection limits (MDLs) in the range of 0.70-2.22, 0.55-3.82, 0.16-5.17, and 0.63-3.96 ng/g wet wt. for OCPRs in fish, Lanchester's freshwater prawn, freshwater snail, and vegetables, respectively. The limit of detections (LODs) and the limit of quantifications (LOQs) were in the range of 0.001-0.05 ng/mL and 0.002-0.20 ng/mL, respectively. We considered the methods

to be reliable to quantify the concentration of OCPRs in those aquatic organisms following the AOAC Peer Verified Methods Program (1993).

6.2.6 Dietary survey

The semi-quantitative questionnaire included 4 organisms categories such as fish, shrimp, freshwater snail, and aquatic vegetable retained from Khlong 7 only (see Appendix F). A questionnaire-based dietary survey was conducted randomly for a number of healthy adults living at Khlong 7 from January to February, 2007. The face-to-face interview (by both the researcher and the community) was focused on the frequency (number of time per day, week, month, or year), quantity of consumption, and body weight. The measuring cup and balance were used during the interview to facilitate the quantification of food intake. Daily consumption (kg/day) for each organism was computed for each individual.

6.2.7 Exposure assessment

An individual exposure to OCPRs from ingesting fish or aquatic organisms (mg/kg-day) was estimated by multiplying the concentration of OCPRs in the edible portion (mg/kg) by mean daily consumption rate (kg/day) before dividing by the average body weight (kg) of surveyed populations (US EPA, 2000b).

6.2.8 Risk characterization

Calculating for carcinogenic toxicity of individual risk (US EPA, 2000b), the lifetime risk was estimated by multiplying the cancer slope factor by exposure data (mg/kg-day). In principal, cancer risk can not exceed 1. Additionally, cancer risk for population risk was calculated by multiplying the number of people in an exposure setting at 10^6 (one in one million) by the lifetime cancer risks. For noncarcinogenic toxicity of individual risk estimation, the comparison of exposure to the reference dose (RfD) indicates the degree to which exposure is greater or less than RfD. When the ratio is equal to or greater than 1 (i.e., when exposure exceeds the RfD), the exposed populations may be at risk.

6.3 Results and Discussions

6.3.1 Edible aquatic organisms

Fifteen aquatic organisms were classified into four groups based on the most consumed aquatic organisms of the local population living in Khlong 7 into: (1) 10 species of fish: *Anabas testudineus*, *Channa striatus*, *Notopterus notopterus*, *Orechromis niloticus*, *Oxyeleotris marmoratus*, *Pristolepis fasciata*, *Puntius altus*, *Puntius gonionotus*, *Trichogaster microlepis*, and *Trichogaster trichopterus*, (2) a species of shrimp: *Macrobrachium lanchesteri*, (3) a species of snail: *Filopaludina mertensi*, and (4) 3 species of vegetables: *Ipomomea aquatica* Forssk., *Neptunia oleracea* Lour., and *Nymphaea lotus* L.

6.3.2 OCPRs in edible aquatic organisms

Table 6.2 and figure 6.2 shows OCPRs concentrations (ng/g wet wt.) determined in fish, freshwater shrimp, freshwater snail, and vegetables. The top four averages of OCPRs, in decreasing order, in all matrices were DDT (24.57 ng/g wet wt.), ∑ endosulfan (20.37 ng/g wet wt.), β-HCH (9.18 ng/g wet wt.), and heptachlor (7.99 ng/g wet wt.). In spite of DDT and its derivatives being prohibited for over 20 years, 4, 4' DDT showed the highest concentration among their derivatives. It is worth noting that there is evidence that DDT is present as an impurity in acaricide (mitecide), known as dicofol, which has been detected at high levels in water and soil samples from agricultural areas of Thailand between 1996-1997 (Anat and Paul, 2000). Currently, the use of ∑ endosulfan for the control of golden apple snail, *Pomacea* sp., is illegal in paddy fields sometimes crop beginning, due to possible accumulation in organisms. Notably, HCHs especially β-HCH was the dominant isomer found in aquatic organisms as reported in Hung *et al.* (2006), this may be related to its resistance to enzymatic degradation (Minth *et al.*, 1999). Likewise, heptachlor is a breakdown product and a component of the pesticide chlordane, which was recently banned for use in 2000. Its residue can be discharged by runoff from the soil around buildings treated for termites into water bodies and can accumulate in aquatic organisms (Thirakhupt *et al.*, 2006a).

From the human viewpoint, the presence of OCPRs in aquatic organisms is of particular concern considering the high potential for accumulation of these compounds in human fatty tissue. In other words, OCPRs can cause a range of adverse effects on humans such as cancer, genetic defects, and acute and chronic injury to the nervous system (Hayes *et al.*, 1971; Bor-Cheng *et al.*, 2000)

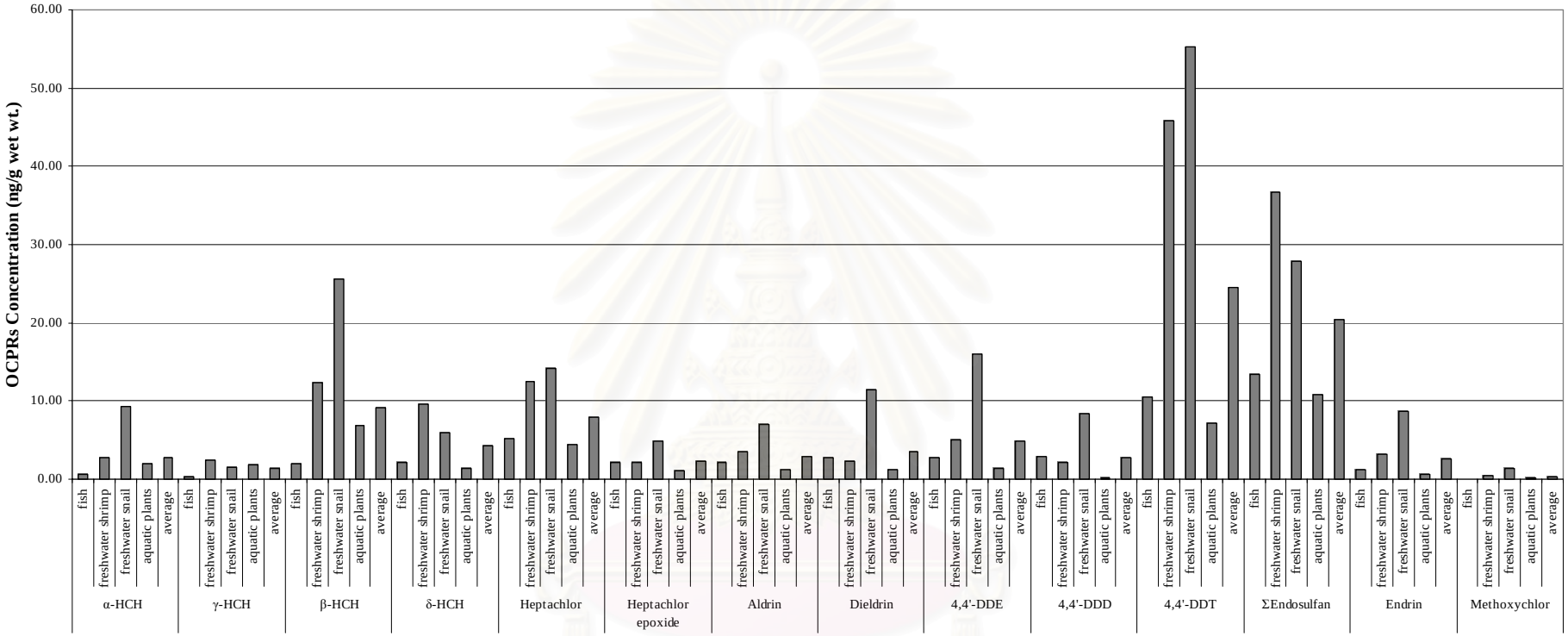


Figure 6.2 Comparison of organochlorine pesticide residues (OCPRs) concentrations (ng/g wet wt.) in fish, freshwater shrimp (Lanchester's freshwater prawn), freshwater snail, and vegetables including the average values of all matrices collected from Khlong 7, Rangsit agricultural area, central Thailand

6.3.3 Risk assessment

6.3.3.1 Dietary survey

Fifty-one of the participants (33 male (65%) and 18 female (35%)) reported that they consumed fish from Khlong 7. The average age (±S.D.) was 36±14 years (range: 10-75) and the average weight (±S.D.) was 59±14 kg (range: 30-120). All participants have been living in Khlong 7 community for 11-15 years. In this study, 15 of the most consumed species were selected for individual dietary consumption interview. The local people reported that when they consumed some groups of fish such as (1) *Anabas testudineus* and *Pristolepis fasciata*, (2) *Puntius gonionotus* and *Puntius altus*, and (3) *Trichogaster microlepis* and *Trichogaster trichopterus*; they did not separate fish into individual species. The average daily consumption of some grouped fish and individual species of fish, shrimp, freshwater snail, and vegetables are given in Table 6.1. The most commonly consumed organisms for fish, shrimp, snail, and vegetables were *Channa striatus* (0.0874 kg/day), *Macrobrachium lanchesteri* (0.0025 kg/day), *Filopaludina mertensi* (0.0080 kg/day), and *Nymphaea lotus* (0.0139 kg/day). Due to the fact that these aquatic organisms are the most consumed species in Khlong 7, people consuming large amounts of these contaminated organisms may have elevated concentration of OCPRs in their tissue compared to the general Thai population (see Table 6.1).

6.3.3.2 Risk characterization

An evaluation of non-carcinogenic and carcinogenic risks to the local population of Khlong 7 was undertaken and is summarized in Table 6.2. Relevant oral reference dose (RFD), and cancer slope factor (CSF) can be obtained from US EPA's Integrated Risk Information System (IRIS), www.epa.gov/iris/. The individual exposures (mg/kg-d) were calculated using the upper bound of 95% confidence interval (C.I.) for OCPRs mean of local populations. It is useful to estimate population risk and establish exposure limits to provide a plausible worst-case scenario for initial screening of potential risk. Moreover, the benchmarks of non-cancer and cancer risk are set at the value 1.0 shown in figure 6.3-6.13. When the calculation of population cancer risk hazardous ratio and non-cancer hazardous ratio is greater than benchmark,

an initiation of appropriate management strategies should be considered. The results indicated that the 9 contaminants, α-, β-HCH, heptachlor, heptachlor epoxide, aldrin, dieldrin, 4,4'-DDE, 4,4'-DDD, and 4,4'-DDT, may be of particular concern for the lifetime risk potential for cancer associated with the consumption of fish from Khlong 7 due to the calculated population cancer risk being greater than 1.0 (benchmark). The consumption of snakehead fish, *Channa striatus*, was the most associated with cancer from dieldrin contamination. Likewise, Lanchester's freshwater prawn (*Macrobrachium lanchesteri*), freshwater snail (*Filopaludina mertensi*), and vegetables such as swamp morning-glory (*Ipomomea aquatica*), neptunia (*Neptunia oleracea*), and water lily (*Nymphaea lotus*) were a cancer causing risk due to α-, β-HCH, heptachlor, heptachlor epoxide, aldrin, and dieldrin contamination. In addition, Jiang *et al.* (2005) indicated that the populations in a coastal city, China were at risk causing from the contamination of HCH, dieldrin, chlordane, DDTs, and PCBs, suggesting that daily exposure to these contaminants due to fish consumption had a lifetime cancer risk of greater than one in one million.

Consumption of snakehead fish contaminated with heptachlor epoxide was shown to be a risk with a benchmark greater than 1.0. IRIS (2007j) reported the critical effects for non-carcinogenic heptachlor epoxide to be an increased liver-to-body weight ratio in both males and females. Endosulfan had a non-cancer hazardous ratio less than 1.0, which may be because it can be broken down within 1 day to several months depending on the water conditions (Thirakhupt *et al.*, 2006a) and its octanol-water partitioning coefficient (K_{ow}= 3.55-3.62) is lower than other OCPs.

Where the risk is higher, various risk management decisions may have to be made by the regulatory authorities in Thailand such as the Ministry of public health and the Ministry of natural resources and environment. However, residue levels in prepared food often get reduced substantially when the raw commodity is subjected to trimming, washing, and cooking (US EPA, 2000b). This may reduce the risk due to the consumption of aquatic organisms' of the local population at Khlong 7.

Table 6.1 Average daily consumption of various aquatic species for the local population (n=51) in Khlong 7, Rangsit agricultural area and general Thai population

Aquatic Organisms[a]	**Average Daily Consumption for Local Population in Khlong 7 (mean ± S.E., kg/day)**	**Average Daily Consumption for Thai**[b] **(mean ± S.D., kg/day)**
Anabas testudineus & *Pristolepis fasciata* (climbing perch & catopra)	0.0143±0.0035	-
Channa striatus (snakehead)	0.0874±0.0148	0.0027±0.0114
Notopterus notopterus (bronze featherback)	0.0064±0.0015	0.0002±0.0024
Orechromis niloticus (Nile tilapia)	0.0375±0.0061	0.0025±0.0137
Oxyeleotris marmoratus (marbled sleeper)	0.0027±0.0010	-
Puntius gonionotus & *Puntius altus* (silver barb & Trey kahe[c])	0.0697±0.0112	0.0013±0.0083
Trichogaster microlepis & *Trichogaster trichopterus* (moonbeam gourami & three-spot gourami)	0.0049±0.0015	0.0004±0.0006
Macrobrachium lanchesteri (Lanchester's freshwater prawn)	0.0025±0.0009	0.0001±0.0017
Filopaludina mertensi (freshwater snail)	0.0080±0.0024	-
Ipomomea aquatica Forssk. (swamp morning-glory)	0.0136±0.0038	0.0018±0.0088
Neptunia oleracea Lour. (neptunia)	0.0072±0.0021	0.0013±0.0076
Nymphaea lotus L (water lily)	0.0139±0.0040	0.0004±0.0042

[a] Identification keys are from Nelson (1976), Rainboth, (1996), Monkolprasit *et al*. (1997), Suwannakul and Suwannakearnikom (2001), Vidthayanon (2002), and Vidthayanon (2004).
[b] Source: Nutrition Division (1995)
[c] local name

Table 6.2 Risk characterizations of organochlorine pesticide residues (OCPRs) in favorite edible aquatic species collected from Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand from June 2004 to May 2005

Organochlorine Pesticides	Aquatic Organisms	n	Daily Consumption Rate Mean [c] (kg/d)	OCPRs Mean ($\times10^{-3}$ mg/kg wet wt.)	Std. Error (S.E.)	95% Confidence Interval for OCPRs Mean ($\times10^{-3}$ mg/kg wet wt.)		Individual Exposure [d] (E_m) (mg/kg-d)	Risk Characterization		
						Lower Bound	Upper Bound		Non Carcinogenic Toxicity [e]	Carcinogenic Toxicity	
										Lifetime Risk [f]	Population Cancer Risk [g]
α-HCH[a]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	ND	ND	ND	ND	ND	-	ND	ND
	Channa striatus♠	9	0.0874	4.34	0.13	4.04	4.64	6.9×10^{-6}	-	4.3×10^{-5}	4.3×10
	Notopterus notopterus♠	15	0.0064	ND	ND	ND	ND	ND	-	ND	ND
	Orechromis niloticus♠	3	0.0375	0.08	0.08	-0.27	0.43	2.7×10^{-7}	-	1.7×10^{-6}	1.7
	Oxyeleotris marmoratus♠	15	0.0027	1.12	0.16	0.78	1.47	6.7×10^{-8}	-	4.2×10^{-7}	4.2×10^{-1}
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	0.31	0.12	0.06	0.56	6.6×10^{-7}	-	4.2×10^{-6}	4.2
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	0.34	0.08	0.17	0.51	4.2×10^{-8}	-	2.7×10^{-7}	2.7×10^{-1}
	Macrobrachium lanchesteri♣	93	0.0025	2.77	0.52	1.74	3.81	1.6×10^{-7}	-	1.0×10^{-6}	1.0
	Filopaludina mertensi♥	57	0.0080	9.24	0.71	7.81	10.68	1.4×10^{-6}	-	9.1×10^{-6}	9.1
	Ipomomea aquatica Forssk.♦	42	0.0136	0.83	0.11	0.61	1.04	2.4×10^{-7}	-	1.5×10^{-6}	1.5
	Neptunia oleracea Lour.♦	6	0.0072	2.70	0.13	2.37	3.04	3.7×10^{-7}	-	2.3×10^{-6}	2.3
	Nymphaea lotus L.♦	57	0.0139	2.68	0.64	1.39	3.96	9.3×10^{-7}	-	5.9×10^{-6}	5.9
γ-HCH[a]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	0.22	0.15	-0.13	0.56	1.4×10^{-7}	5×10^{-4}	-	-
	Channa striatus♠	9	0.0874	ND	ND	ND	ND	ND	ND	-	-
	Notopterus notopterus♠	15	0.0064	ND	ND	ND	ND	ND	ND	-	-
	Orechromis niloticus♠	3	0.0375	1.55	0.06	1.29	1.80	1.1×10^{-6}	4×10^{-3}	-	-
	Oxyeleotris marmoratus♠	15	0.0027	ND	ND	ND	ND	ND	ND	-	-
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	0.24	0.09	0.06	0.42	5.0×10^{-7}	2×10^{-3}	-	-
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	0.35	0.07	0.21	0.49	4.1×10^{-8}	1×10^{-4}	-	-

Organochlorine Pesticides	Aquatic Organisms	n	Daily Consumption Rate Mean [c] (kg/d)	OCPRs Mean ($\times10^{-3}$ mg/kg wet wt.)	Std. Error (S.E.)	95% Confidence Interval for OCPRs Mean ($\times10^{-3}$ mg/kg wet wt.)		Individual Exposure [d] (E_m) (mg/kg-d)	Risk Characterization		
						Lower Bound	Upper Bound		Non Carcinogenic Toxicity [e]	Carcinogenic Toxicity: Lifetime Risk [f]	Carcinogenic Toxicity: Population Cancer Risk [g]
	Macrobrachium lanchesteri♣	93	0.0025	2.40	0.34	1.73	3.07	1.3×10^{-7}	4×10^{-4}	-	-
	Filopaludina mertensi♥	57	0.0080	1.52	0.56	0.40	2.64	3.6×10^{-7}	1×10^{-3}	-	-
	Ipomomea aquatica Forssk.♦	42	0.0136	1.23	0.21	0.81	1.65	3.8×10^{-7}	1×10^{-3}	-	-
	Neptunia oleracea Lour.♦	6	0.0072	4.52	0.07	4.34	4.70	5.7×10^{-7}	2×10^{-3}	-	-
	Nymphaea lotus L.♦	57	0.0139	2.02	0.28	1.47	2.58	6.1×10^{-7}	2×10^{-3}	-	-
β-HCH[a]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	2.48	0.89	0.43	4.53	1.1×10^{-6}	-	2.0×10^{-6}	2.0
	Channa striatus♠	9	0.0874	5.17	0.41	4.22	6.13	9.1×10^{-6}	-	1.6×10^{-5}	1.6×10
	Notopterus notopterus♠	15	0.0064	0.80	0.18	0.42	1.18	1.3×10^{-7}	-	2.3×10^{-7}	2.3×10^{-1}
	Orechromis niloticus♠	3	0.0375	2.47	0.07	2.18	2.76	1.8×10^{-6}	-	3.2×10^{-6}	3.2
	Oxyeleotris marmoratus♠	15	0.0027	1.97	0.09	1.76	2.17	9.9×10^{-8}	-	1.8×10^{-7}	1.8×10^{-1}
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	1.11	0.25	0.61	1.62	1.9×10^{-6}	-	3.4E-06	3.4
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	2.20	0.35	1.49	2.92	2.4×10^{-7}	-	4.4×10^{-7}	4.4×10^{-1}
	Macrobrachium lanchesteri♣	93	0.0025	12.31	1.13	10.06	14.56	6.2×10^{-7}	-	1.1×10^{-6}	1.1
	Filopaludina mertensi♥	57	0.0080	25.57	2.89	19.78	31.37	4.3×10^{-6}	-	7.7×10^{-6}	7.7
	Ipomomea aquatica Forssk.♦	42	0.0136	3.50	0.13	3.22	3.77	8.7×10^{-7}	-	1.6×10^{-6}	1.6
	Neptunia oleracea Lour.♦	6	0.0072	29.26	6.71	12.01	46.51	5.7×10^{-6}	-	1.0×10^{-5}	1.0×10
	Nymphaea lotus L.♦	57	0.0139	6.91	0.65	5.62	8.21	1.9×10^{-6}	-	3.5×10^{-6}	3.5
Heptachlor[a,b]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	4.81	1.30	1.82	7.81	1.9×10^{-6}	4×10^{-3}	8.5×10^{-6}	8.5
	Channa striatus♠	9	0.0874	17.03	0.44	16.03	18.04	2.7×10^{-5}	5×10^{-2}	1.2×10^{-4}	1.2×10^{2}
	Notopterus notopterus♠	15	0.0064	0.98	0.32	0.29	1.68	1.8×10^{-7}	4×10^{-4}	8.2×10^{-7}	8.2×10^{-1}
	Orechromis niloticus♠	3	0.0375	6.16	0.07	5.87	6.45	4.1×10^{-6}	8×10^{-3}	1.8×10^{-5}	1.8×10
	Oxyeleotris marmoratus♠	15	0.0027	12.52	1.55	9.2	15.84	7.2×10^{-7}	1×10^{-3}	3.3×10^{-6}	3.3
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	2.69	0.29	2.1	3.29	3.9×10^{-6}	8×10^{-3}	1.7×10^{-5}	1.7×10

Organochlorine Pesticides	Aquatic Organisms	n	Daily Consumption Rate Mean [c] (kg/d)	OCPRs Mean ($\times10^{-3}$ mg/kg wet wt.)	Std. Error (S.E.)	95% Confidence Interval for OCPRs Mean ($\times10^{-3}$ mg/kg wet wt.)		Individual Exposure [d] (E_m) (mg/kg-d)	Risk Characterization		
						Lower Bound	Upper Bound		Non Carcinogenic Toxicity [e]	Carcinogenic Toxicity: Lifetime Risk [f]	Carcinogenic Toxicity: Population Cancer Risk [g]
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	3.83	0.38	3.07	4.59	3.8×10^{-7}	8×10^{-4}	1.7×10^{-6}	1.7
	Macrobrachium lanchesteri♣	93	0.0025	12.44	0.76	10.93	13.95	5.9×10^{-7}	1×10^{-3}	2.7×10^{-6}	2.7
	Filopaludina mertensi♥	57	0.0080	14.12	1.24	11.64	16.60	2.3×10^{-6}	5×10^{-3}	1.0×10^{-5}	1.0×10
	Ipomomea aquatica Forssk.♦	42	0.0136	4.29	0.15	3.99	4.58	1.1×10^{-6}	2×10^{-3}	4.8×10^{-6}	4.8
	Neptunia oleracea Lour.♦	6	0.0072	3.39	1.52	-0.51	7.29	8.9×10^{-7}	2×10^{-3}	4.0×10^{-6}	4.0
	Nymphaea lotus L.♦	57	0.0139	4.48	0.24	4.00	4.97	1.2×10^{-6}	2×10^{-3}	5.3×10^{-6}	5.3
Heptachlor epoxide[a,b]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	0.52	0.16	0.16	0.88	2.1×10^{-7}	1.6×10^{-2}	1.9×10^{-6}	1.9
	Channa striatus♠	9	0.0874	11.61	0.2	11.15	12.07	1.8×10^{-5}	1.4	1.6×10^{-4}	1.6×10^{-2}
	Notopterus notopterus♠	15	0.0064	0.16	0.08	-0.02	0.34	3.7×10^{-8}	2.8×10^{-3}	3.4×10^{-7}	3.4×10^{-1}
	Orechromis niloticus♠	3	0.0375	ND	ND	ND	ND	ND	ND	ND	ND
	Oxyeleotris marmoratus♠	15	0.0027	8.80	1.2	6.22	11.38	5.2×10^{-7}	4.0×10^{-2}	4.7×10^{-6}	4.7
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	0.51	0.16	0.20	0.83	9.8×10^{-7}	7.5×10^{-2}	8.9×10^{-6}	8.9
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	0.77	0.11	0.55	0.99	8.2×10^{-8}	6.3×10^{-3}	7.5×10^{-7}	7.5×10^{-1}
	Macrobrachium lanchesteri♣	93	0.0025	2.09	0.37	1.36	2.81	1.2×10^{-7}	9.2×10^{-3}	1.1×10^{-6}	1.1
	Filopaludina mertensi♥	57	0.0080	4.80	1.78	1.24	8.36	1.1×10^{-6}	8.7×10^{-2}	1.0×10^{-5}	1.0×10
	Ipomomea aquatica Forssk.♦	42	0.0136	1.11	0.17	0.77	1.44	3.3×10^{-7}	2.6×10^{-2}	3.0×10^{-6}	3.0
	Neptunia oleracea Lour.♦	6	0.0072	0.80	0.36	-0.12	1.72	2.1×10^{-7}	1.6×10^{-2}	1.9×10^{-6}	1.9
	Nymphaea lotus L.♦	57	0.0139	1.16	0.15	0.86	1.46	3.4×10^{-7}	2.6×10^{-2}	3.1×10^{-6}	3.1
Aldrin[a,b]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	1.58	0.65	0.09	3.07	7.4×10^{-7}	2×10^{-2}	1.3×10^{-5}	1.3×10
	Channa striatus♠	9	0.0874	8.83	0.11	8.57	9.09	1.3×10^{-5}	4×10^{-1}	2.3×10^{-4}	2.3×10^{2}
	Notopterus notopterus♠	15	0.0064	0.47	0.08	0.31	0.63	6.8×10^{-8}	2×10^{-3}	1.2×10^{-6}	1.2
	Orechromis niloticus♠	3	0.0375	2.03	0.13	1.49	2.58	1.6×10^{-6}	5×10^{-2}	2.8×10^{-5}	2.8×10
	Oxyeleotris marmoratus♠	15	0.0027	5.14	0.61	3.83	6.45	3.0×10^{-7}	1×10^{-2}	5.0×10^{-6}	5.0

Organochlorine Pesticides	Aquatic Organisms	n	Daily Consumption Rate Mean [c] (kg/d)	OCPRs Mean ($\times10^{-3}$ mg/kg wet wt.)	Std. Error (S.E.)	95% Confidence Interval for OCPRs Mean ($\times10^{-3}$ mg/kg wet wt.)		Individual Exposure [d] (E_m) (mg/kg-d)	Risk Characterization		
						Lower Bound	Upper Bound		Non Carcinogenic Toxicity [e]	Carcinogenic Toxicity: Lifetime Risk [f]	Carcinogenic Toxicity: Population Cancer Risk [g]
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	0.37	0.08	0.21	0.53	6.3×10^{-7}	2×10^{-2}	1.1×10^{-5}	1.1×10
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	1.69	0.20	1.29	2.09	1.7×10^{-7}	6×10^{-3}	3.0×10^{-6}	3.0
	Macrobrachium lanchesteri♣	93	0.0025	3.52	0.28	2.96	4.09	1.7×10^{-7}	6×10^{-3}	2.9×10^{-6}	2.9
	Filopaludina mertensi♥	57	0.0080	6.93	0.53	5.88	7.98	1.1×10^{-6}	4×10^{-2}	1.8×10^{-5}	1.8×10
	Ipomomea aquatica Forssk.♦	42	0.0136	1.84	0.74	0.35	3.33	7.7×10^{-7}	3×10^{-2}	1.3×10^{-5}	1.3×10
	Neptunia oleracea Lour.♦	6	0.0072	2.23	0.45	1.07	3.39	4.1×10^{-7}	1×10^{-2}	7.0×10^{-6}	7.0
	Nymphaea lotus L.♦	57	0.0139	0.63	0.09	0.46	0.8	1.9×10^{-7}	6×10^{-3}	3.2×10^{-6}	3.2
Dieldrin[a,b]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	1.66	0.49	0.53	2.78	6.7×10^{-7}	1×10^{-2}	1.1×10^{-5}	1.1×10
	Channa striatus♠	9	0.0874	13.62	0.19	13.18	14.05	2.1×10^{-5}	4×10^{-1}	3.3×10^{-4}	3.3×10^{2}
	Notopterus notopterus♠	15	0.0064	0.39	0.10	0.16	0.61	6.6×10^{-8}	1×10^{-3}	1.1×10^{-6}	1.1
	Orechromis niloticus♠	3	0.0375	ND	ND	ND	ND	ND	ND	ND	ND
	Oxyeleotris marmoratus♠	15	0.0027	10.78	1.33	7.93	13.63	6.2×10^{-7}	1×10^{-2}	1.0×10^{-5}	1.0×10
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	0.37	0.21	-0.05	0.8	9.5×10^{-7}	2×10^{-2}	1.5×10^{-5}	1.5×10
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	0.98	0.42	0.15	1.82	1.5×10^{-7}	3×10^{-3}	2.4×10^{-6}	2.4
	Macrobrachium lanchesteri♣	93	0.0025	2.36	0.38	1.61	3.11	1.3×10^{-7}	3×10^{-3}	2.1×10^{-6}	2.1
	Filopaludina mertensi♥	57	0.0080	11.4	1.24	8.92	13.88	1.9×10^{-6}	4×10^{-2}	3.0×10^{-5}	3.0×10
	Ipomomea aquatica Forssk.♦	42	0.0136	1.04	0.10	0.84	1.25	2.9×10^{-7}	6×10^{-3}	4.6×10^{-6}	4.6
	Neptunia oleracea Lour.♦	6	0.0072	0.68	0.34	-0.20	1.55	1.9×10^{-7}	4×10^{-3}	3.0×10^{-6}	3.0
	Nymphaea lotus L.♦	57	0.0139	1.51	0.21	1.09	1.94	4.6×10^{-7}	9×10^{-3}	7.3×10^{-6}	7.3
Endrin[b]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	1.23	0.34	0.45	2	4.8×10^{-7}	2×10^{-3}	-	-
	Channa striatus♠	9	0.0874	ND	ND	ND	ND	ND	ND	-	-
	Notopterus notopterus♠	15	0.0064	0.23	0.06	0.10	0.36	3.9×10^{-8}	1×10^{-4}	-	-
	Orechromis niloticus♠	3	0.0375	3.83	0.09	3.45	4.2	2.7×10^{-6}	9×10^{-3}	-	-

Organochlorine Pesticides	Aquatic Organisms	n	Daily Consumption Rate Mean [c] (kg/d)	OCPRs Mean ($\times 10^{-3}$ mg/kg wet wt.)	Std. Error (S.E.)	95% Confidence Interval for OCPRs Mean ($\times 10^{-3}$ mg/kg wet wt.)		Individual Exposure [d] (E_m) (mg/kg-d)	Risk Characterization		
						Lower Bound	Upper Bound		Non Carcinogenic Toxicity [e]	Carcinogenic Toxicity: Lifetime Risk [f]	Carcinogenic Toxicity: Population Cancer Risk [g]
	Oxyeleotris marmoratus♠	15	0.0027	0.27	0.27	-0.31	0.84	3.8×10^{-8}	1×10^{-4}	-	-
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	0.47	0.19	0.08	0.87	1.0×10^{-6}	3×10^{-3}	-	-
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	2.19	0.27	1.64	2.74	2.3×10^{-7}	8×10^{-4}	-	-
	Macrobrachium lanchesteri♣	93	0.0025	3.17	0.72	1.73	4.61	2.0×10^{-7}	7×10^{-4}	-	-
	Filopaludina mertensi♥	57	0.0080	8.65	1.57	5.52	11.79	1.6×10^{-6}	5×10^{-3}	-	-
	Ipomomea aquatica Forssk.♦	42	0.0136	0.71	0.22	0.27	1.14	2.6×10^{-7}	9×10^{-4}	-	-
	Neptunia oleracea Lour.♦	6	0.0072	ND	ND	ND	ND	ND	ND	-	-
	Nymphaea lotus L.♦	57	0.0139	0.48	0.13	0.21	0.75	1.8×10^{-7}	6×10^{-4}	-	-
4,4'-DDE[a]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	2.01	0.34	1.22	2.80	6.8×10^{-7}	-	2.3×10^{-7}	2.3×10^{-1}
	Channa striatus♠	9	0.0874	11.51	0.42	10.54	12.49	1.9×10^{-5}	-	6.3×10^{-6}	6.3
	Notopterus notopterus♠	15	0.0064	0.88	0.08	0.71	1.06	1.1×10^{-7}	-	3.9×10^{-8}	3.9×10^{-2}
	Orechromis niloticus♠	3	0.0375	1.42	0.11	0.94	1.89	1.2×10^{-6}	-	4.1×10^{-7}	4.1×10^{-1}
	Oxyeleotris marmoratus♠	15	0.0027	5.91	0.74	4.33	7.49	3.4×10^{-7}	-	1.2×10^{-7}	1.2×10^{-1}
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	1.21	0.20	0.81	1.62	1.9×10^{-6}	-	6.5×10^{-7}	6.5×10^{-1}
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	2.02	0.30	1.41	2.63	2.2×10^{-7}	-	7.4×10^{-8}	7.4×10^{-2}
	Macrobrachium lanchesteri♣	93	0.0025	5.04	0.41	4.23	5.84	2.5×10^{-7}	-	8.4×10^{-8}	8.4×10^{-2}
	Filopaludina mertensi♥	57	0.0080	15.93	1.14	13.64	18.23	2.5×10^{-6}	-	8.4×10^{-7}	8.4×10^{-1}
	Ipomomea aquatica Forssk.♦	42	0.0136	1.17	0.19	0.79	1.54	3.5×10^{-7}	-	1.2×10^{-7}	1.2×10^{-1}
	Neptunia oleracea Lour.♦	6	0.0072	4.98	0.24	4.37	5.60	6.8×10^{-7}	-	2.3×10^{-7}	2.3×10^{-1}
	Nymphaea lotus L.♦	57	0.0139	1.05	0.16	0.72	1.38	3.3×10^{-7}	-	1.1×10^{-7}	1.1×10^{-1}
4,4'-DDD[a]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	1.42	0.45	0.38	2.47	6.0×10^{-7}	-	1.4×10^{-7}	1.4×10^{-1}
	Channa striatus♠	9	0.0874	12.3	0.3	11.61	12.98	1.9×10^{-5}	-	4.6×10^{-6}	4.6
	Notopterus notopterus♠	15	0.0064	0.17	0.09	-0.02	0.35	3.8×10^{-8}	-	9.1×10^{-9}	9.1×10^{-3}

Organochlorine Pesticides	Aquatic Organisms	n	Daily Consumption Rate Mean [c] (kg/d)	OCPRs Mean ($\times10^{-3}$ mg/kg wet wt.)	Std. Error (S.E.)	95% Confidence Interval for OCPRs Mean ($\times10^{-3}$ mg/kg wet wt.)		Individual Exposure [d] (E_m) (mg/kg-d)	Risk Characterization		
						Lower Bound	Upper Bound		Non Carcinogenic Toxicity [e]	Carcinogenic Toxicity	
										Lifetime Risk [f]	Population Cancer Risk [g]
	Orechromis niloticus♠	3	0.0375	3.72	0.08	3.36	4.07	2.6×10^{-8}	-	6.2×10^{-7}	6.2×10^{-1}
	Oxyeleotris marmoratus♠	15	0.0027	9.12	1.22	6.50	11.74	5.4×10^{-7}	-	1.3×10^{-7}	1.3×10^{-1}
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	0.83	0.27	0.29	1.37	1.6×10^{-6}	-	3.9×10^{-7}	3.9×10^{-1}
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	1.59	0.25	1.09	2.08	1.7×10^{-7}	-	4.1×10^{-8}	4.1×10^{-2}
	Macrobrachium lanchesteri♣	93	0.0025	2.18	0.55	1.08	3.29	1.4×10^{-7}	-	3.3×10^{-8}	3.3×10^{-2}
	Filopaludina mertensi♥	57	0.0080	8.44	2.25	3.93	12.95	1.8×10^{-6}	-	4.2×10^{-7}	4.2×10^{-1}
	Ipomomea aquatica Forssk.♦	42	0.0136	ND	ND	ND	ND	ND	-	ND	ND
	Neptunia oleracea Lour.♦	6	0.0072	3.10	0.27	2.41	3.79	4.6×10^{-7}	-	1.1×10^{-7}	1.1×10^{-1}
	Nymphaea lotus L.♦	57	0.0139	0.02	0.01	ND	0.04	9.4×10^{-9}	-	2.3×10^{-9}	2.3×10^{-3}
4,4'-DDT[a,b]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	5.16	0.97	2.92	7.41	1.8×10^{-6}	4×10^{-3}	6.1×10^{-7}	6.1×10^{-1}
	Channa striatus♠	9	0.0874	33.85	0.89	31.79	35.91	5.3×10^{-5}	1×10^{-1}	1.8E-05	1.8×10
	Notopterus notopterus♠	15	0.0064	4.14	0.47	3.13	5.16	5.6×10^{-7}	1×10^{-3}	1.9×10^{-7}	1.9×10^{-1}
	Orechromis niloticus♠	3	0.0375	10.28	0.07	1ND	10.56	6.7×10^{-6}	1×10^{-2}	2.3×10^{-6}	2.3×10
	Oxyeleotris marmoratus♠	15	0.0027	10.68	1.98	6.43	14.94	6.8×10^{-7}	1×10^{-3}	2.3×10^{-7}	2.3×10^{-1}
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	2.40	0.27	1.85	2.95	3.5×10^{-6}	7×10^{-3}	1.2×10^{-6}	1.2
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	14.6	1.61	11.35	17.85	1.5×10^{-6}	3×10^{-3}	5.0×10^{-7}	5.0×10^{-1}
	Macrobrachium lanchesteri♣	93	0.0025	45.82	7.73	30.47	61.16	2.6×10^{-6}	5×10^{-3}	8.8×10^{-7}	8.8×10^{-1}
	Filopaludina mertensi♥	57	0.0080	55.24	6.56	42.09	68.39	9.3×10^{-6}	2×10^{-2}	3.2×10^{-6}	3.2
	Ipomomea aquatica Forssk.♦	42	0.0136	5.77	0.51	4.74	6.80	1.6×10^{-6}	3×10^{-3}	5.3×10^{-7}	5.3×10^{-1}
	Neptunia oleracea Lour.♦	6	0.0072	11.53	1.90	6.64	16.42	2.0×10^{-6}	4×10^{-3}	6.8×10^{-7}	6.8×10^{-1}
	Nymphaea lotus L.♦	57	0.0139	7.80	1.31	5.17	10.43	2.5×10^{-6}	5×10^{-3}	8.4×10^{-7}	8.4×10^{-1}
Endosulfan[b]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	18.15	4.64	7.46	28.85	7.0×10^{-6}	1×10^{-3}	-	-
	Channa striatus♠	9	0.0874	46.22	0.67	44.67	47.76	7.1×10^{-5}	1×10^{-2}	-	-

Organochlorine Pesticides	Aquatic Organisms	n	Daily Consumption Rate Mean [c] (kg/d)	OCPRs Mean ($\times 10^{-3}$ mg/kg wet wt.)	Std. Error (S.E.)	95% Confidence Interval for OCPRs Mean ($\times 10^{-3}$ mg/kg wet wt.)		Individual Exposure [d] (E_m) (mg/kg-d)	Risk Characterization		
						Lower Bound	Upper Bound		Non Carcinogenic Toxicity [e]	Carcinogenic Toxicity: Lifetime Risk [f]	Carcinogenic Toxicity: Population Cancer Risk [g]
	Notopterus notopterus♠	15	0.0064	6.97	1.02	4.78	9.16	9.9×10^{-7}	2×10^{-4}	-	-
	Orechromis niloticus♠	3	0.0375	17.71	0.13	17.17	18.25	1.2×10^{-5}	2×10^{-3}	-	-
	Oxyeleotris marmoratus♠	15	0.0027	32.72	3.82	24.54	40.91	1.9×10^{-6}	3×10^{-4}	-	-
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	3.18	0.44	2.28	4.08	4.8×10^{-6}	8×10^{-4}	-	-
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	9.85	0.94	7.96	11.74	9.8×10^{-7}	2×10^{-4}	-	-
	Macrobrachium lanchesteri♣	93	0.0025	36.69	5.71	25.35	48.02	2.0×10^{-6}	3×10^{-4}	-	-
	Filopaludina mertensi♥	57	0.0080	27.87	3.44	20.98	34.76	4.7×10^{-6}	8×10^{-4}	-	-
	Ipomomea aquatica Forssk.♦	42	0.0136	13.79	1.47	10.81	16.76	3.9×10^{-6}	6×10^{-4}	-	-
	Neptunia oleracea Lour.♦	6	0.0072	14.03	0.29	13.29	14.77	1.8×10^{-6}	3×10^{-4}	-	-
	Nymphaea lotus L.♦	57	0.0139	8.22	0.86	6.49	9.95	2.3×10^{-6}	4×10^{-4}	-	-
Methoxychlor[b]	*Anabas testudineus*♠ & *Pristolepis fasciata*♠	9	0.0143	ND	ND	ND	ND	ND	ND	-	-
	Channa striatus♠	9	0.0874	ND	ND	ND	ND	ND	ND	-	-
	Notopterus notopterus♠	15	0.0064	ND	ND	ND	ND	ND	ND	-	-
	Orechromis niloticus♠	3	0.0375	ND	ND	ND	ND	ND	ND	-	-
	Oxyeleotris marmoratus♠	15	0.0027	ND	ND	ND	ND	ND	ND	-	-
	Puntius gonionotus & *Puntius altus*♠	33	0.0697	0.03	0.03	-0.04	0.10	1.2×10^{-7}	2×10^{-5}	-	-
	Trichogaster microlepis♠ & *Trichogaster trichopterus*♠	48	0.0049	ND	ND	ND	ND	ND	ND	-	-
	Macrobrachium lanchesteri♣	93	0.0025	0.52	0.15	0.23	0.81	3.4×10^{-8}	7×10^{-6}	-	-
	Filopaludina mertensi♥	57	0.0080	1.35	0.27	0.82	1.88	2.5×10^{-7}	5×10^{-5}	-	-
	Ipomomea aquatica Forssk.♦	42	0.0136	0.34	0.11	0.12	0.55	1.3×10^{-7}	3×10^{-5}	-	-
	Neptunia oleracea Lour.♦	6	0.0072	ND	ND	ND	ND	ND	ND	-	-
	Nymphaea lotus L.♦	57	0.0139	0.09	0.02	0.04	0.13	3.1×10^{-8}	6×10^{-6}	-	-

See notes at page 95

a - Carcinogenic Toxicity
b - Non Carcinogenic Toxicity
c - Calculated based on 51 interviewed local populations (N=51) who eat only fish caught from Khlong 7, Rangsit Agricultural Area, Pathum Thani Province, Thailand
d - Calculated using upper bound of 95% Confidence Interval (C.I.) of OCPRs mean. This is very useful in estimating population risk and establishing exposure limits because they provide a plausible worst-case scenario.
e - Equals the exposure (E_m) divided by reference dose (RFD)
f - Equals the exposure (E_m) multiplied by the cancer slope factor (CSF)
g - Equals the lifetime risk multiplied by the size of exposed population (10^6, one in one million)
ND - Non Detectable (< Limit of Detection, LOD)
♠,♣,♥,♦ - Fish, Lanchester's Freshwater prawn, Freshwater snail, and vegetables, respectively.

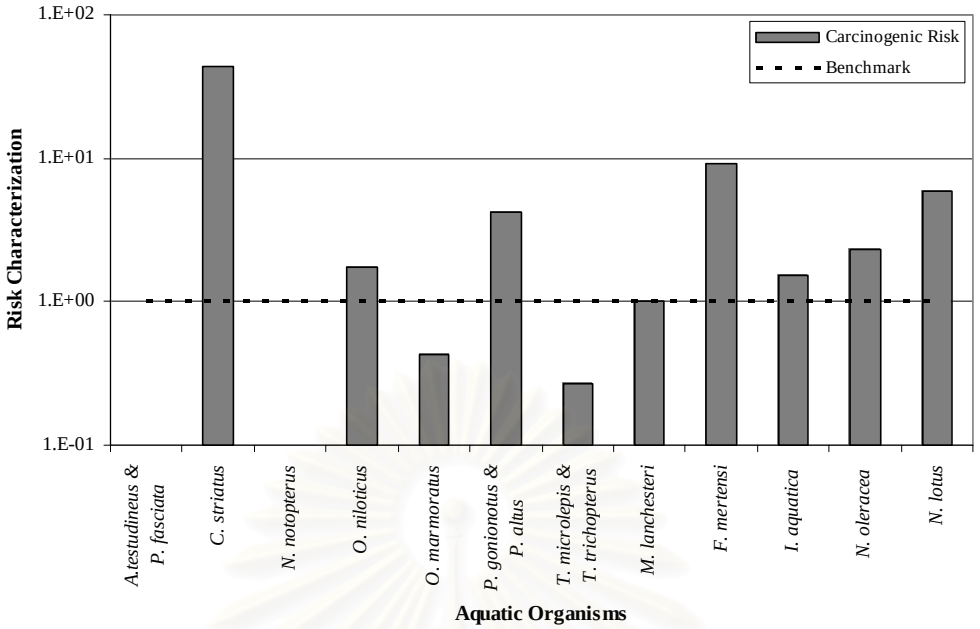


Figure 6.3 Cancer hazardous ratios of α-HCH for daily aquatic organisms' consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

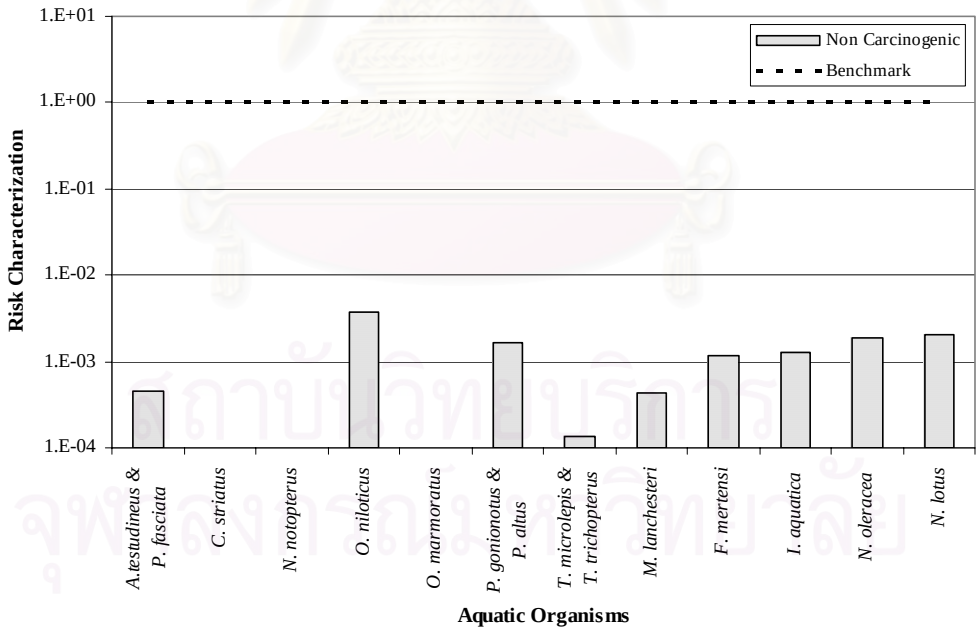


Figure 6.4 Non cancer hazardous ratios of γ-HCH for daily aquatic organisms' consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

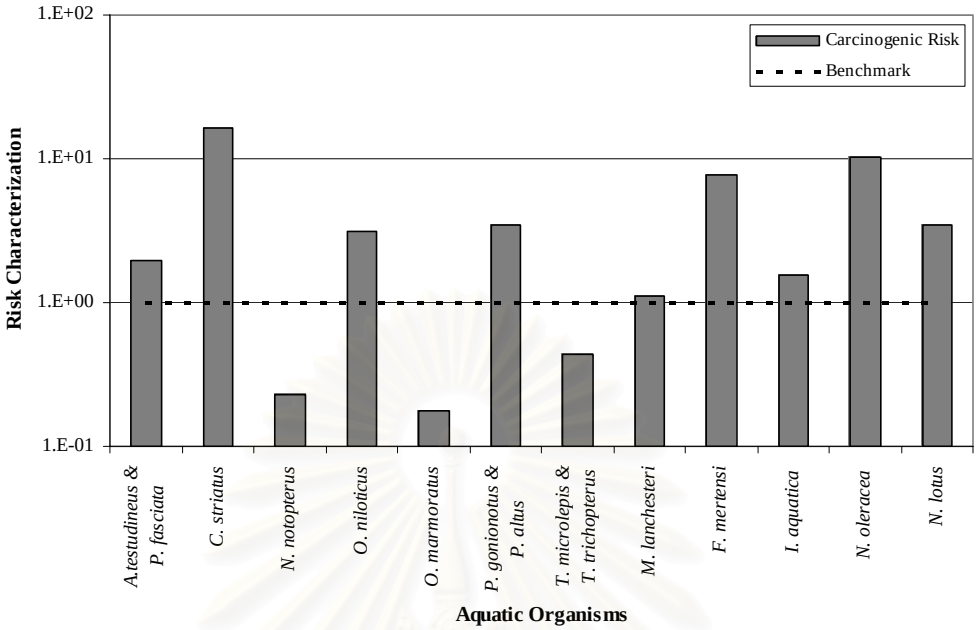


Figure 6.5 Cancer hazardous ratios of β-HCH for daily aquatic organisms' consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

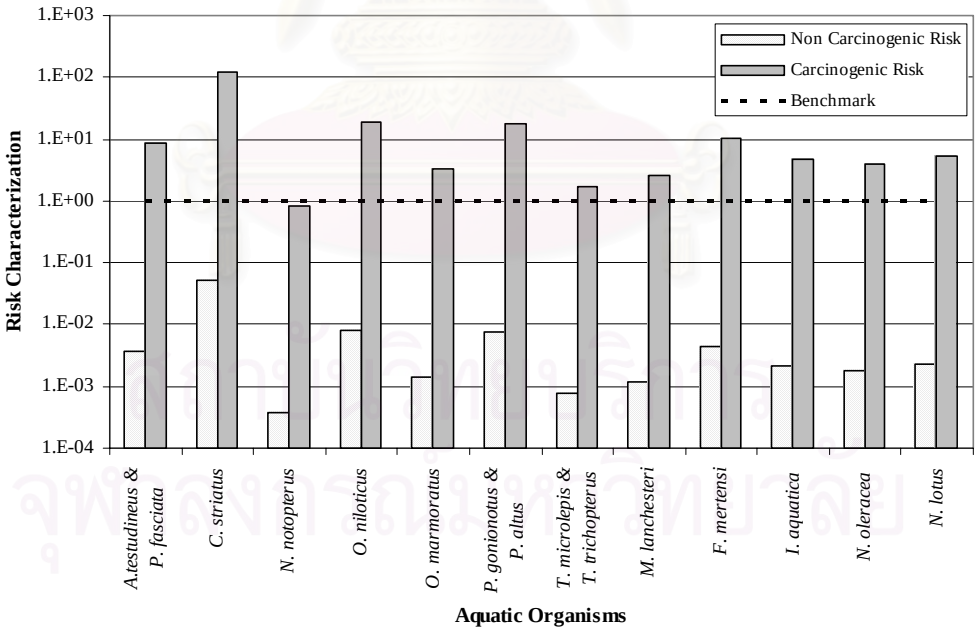


Figure 6.6 Cancer hazardous and non cancer ratios of heptachlor for daily aquatic organisms' consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

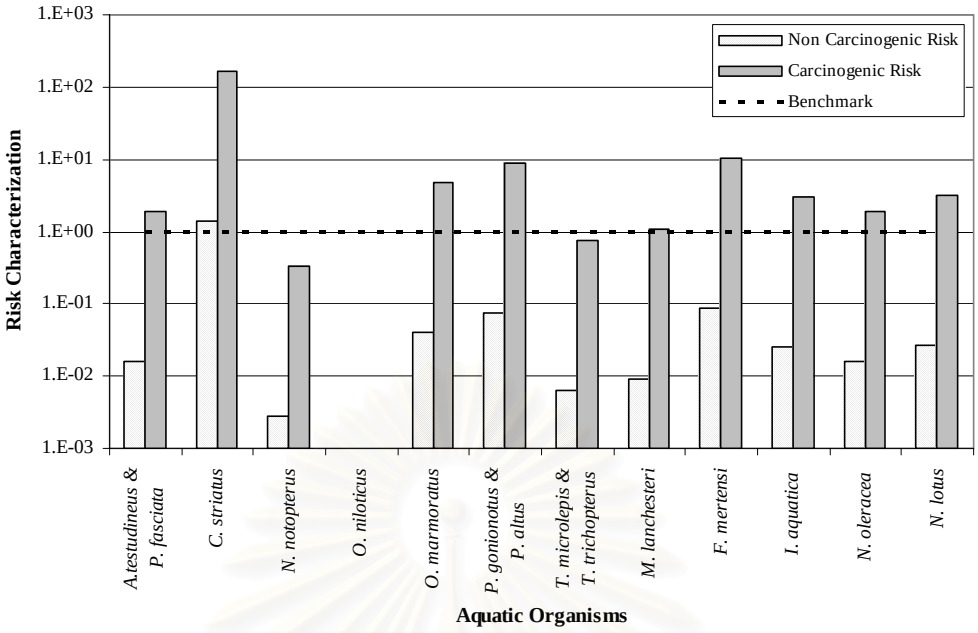


Figure 6.7 Cancer hazardous and non cancer ratios of heptachlor epoxide for daily aquatic organisms' consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

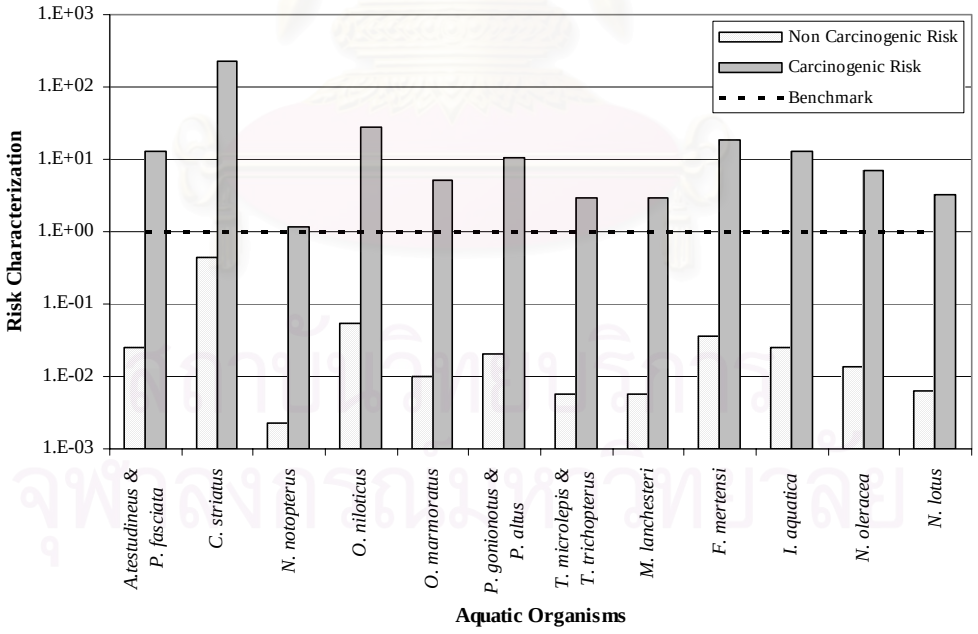


Figure 6.8 Cancer hazardous and non cancer ratios of aldrin for daily aquatic organisms consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

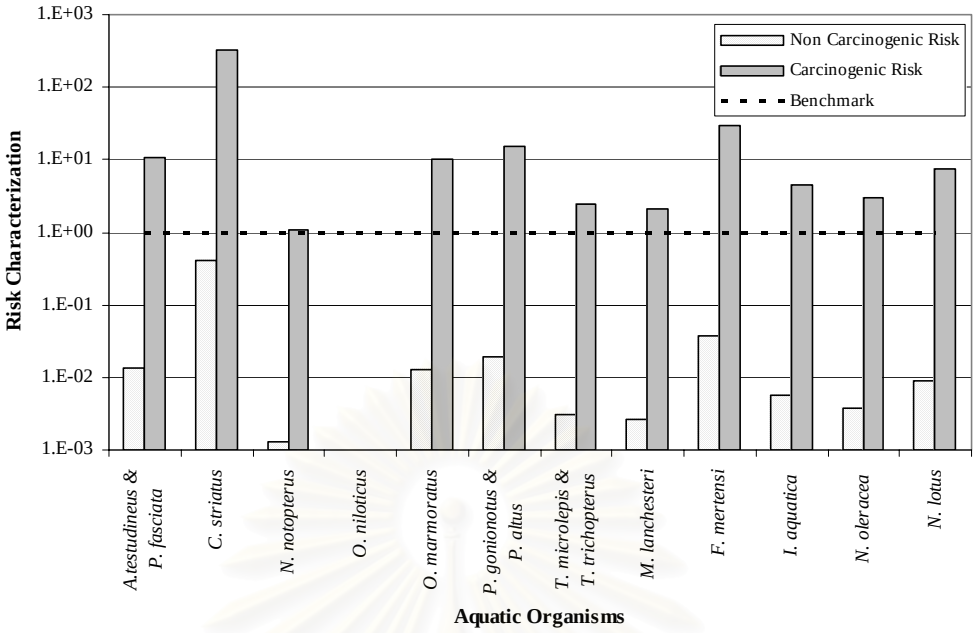


Figure 6.9 Cancer hazardous and non cancer ratios of dieldrin for daily aquatic organisms consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

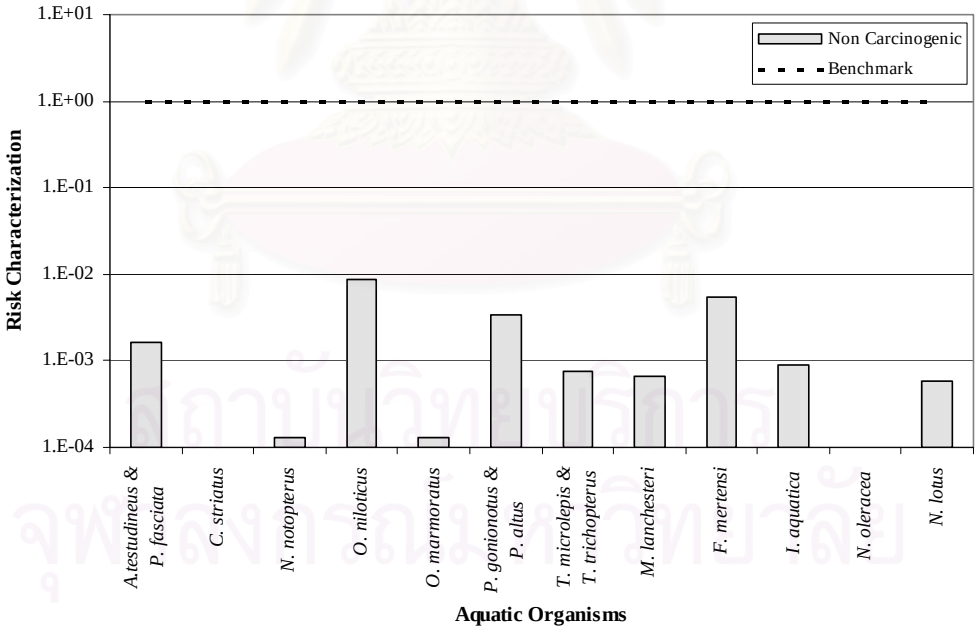


Figure 6.10 Non cancer hazardous ratios of endrin for daily aquatic organisms consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

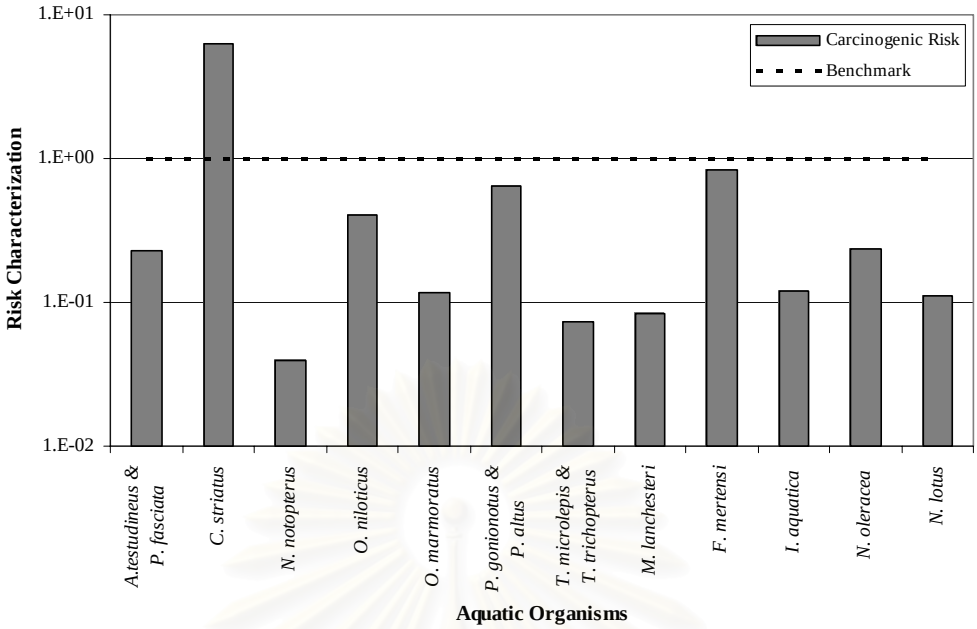


Figure 6.11 Cancer hazardous ratios of 4,4'- DDE for daily aquatic organisms' consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

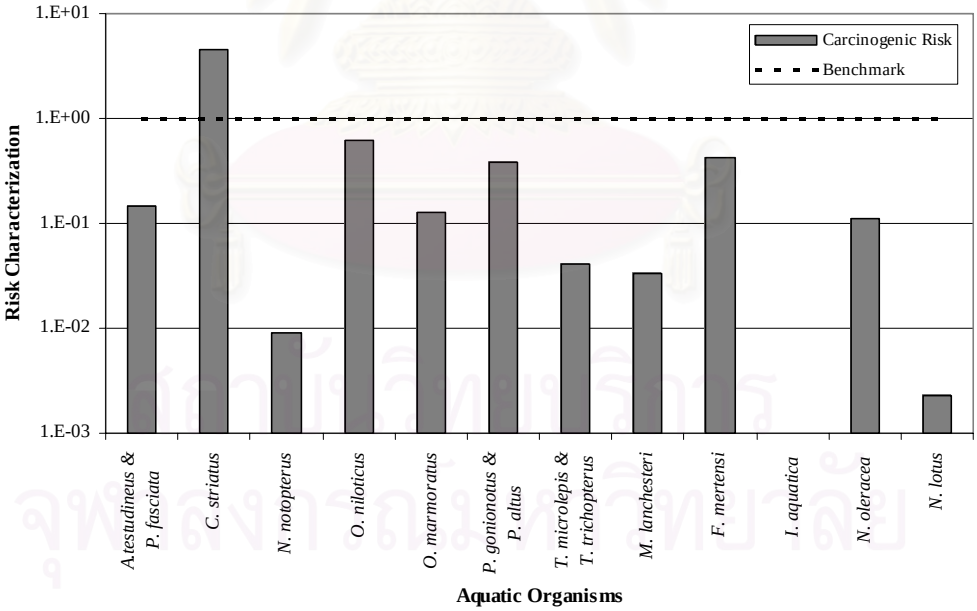


Figure 6.12 Cancer hazardous ratios of 4,4'- DDD for daily aquatic organisms' consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

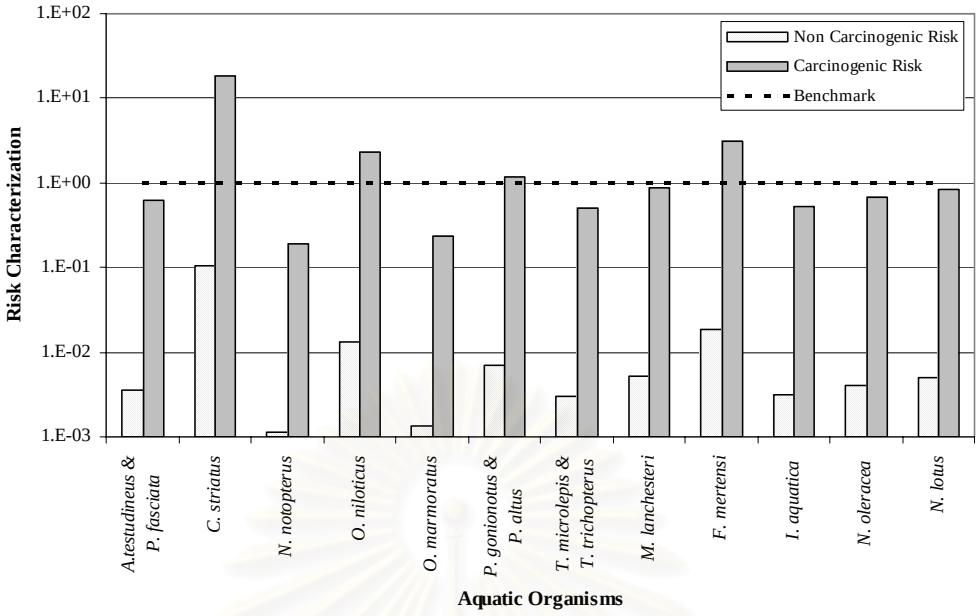


Figure 6.13 Cancer hazardous and non cancer ratios of 4,4'- DDT for daily aquatic organisms' consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

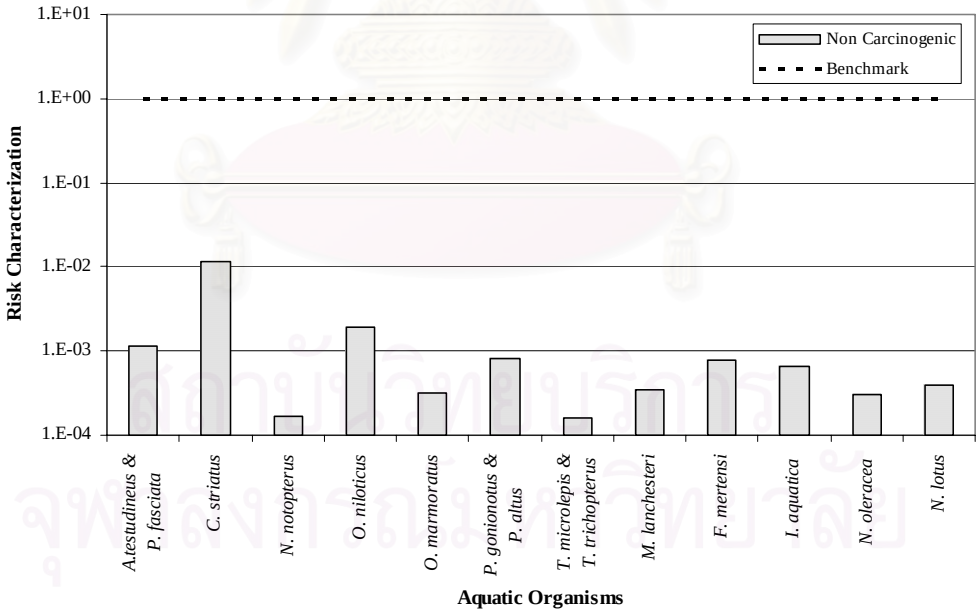


Figure 6.14 Non cancer hazardous ratios of endosulfan for daily aquatic organisms' consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

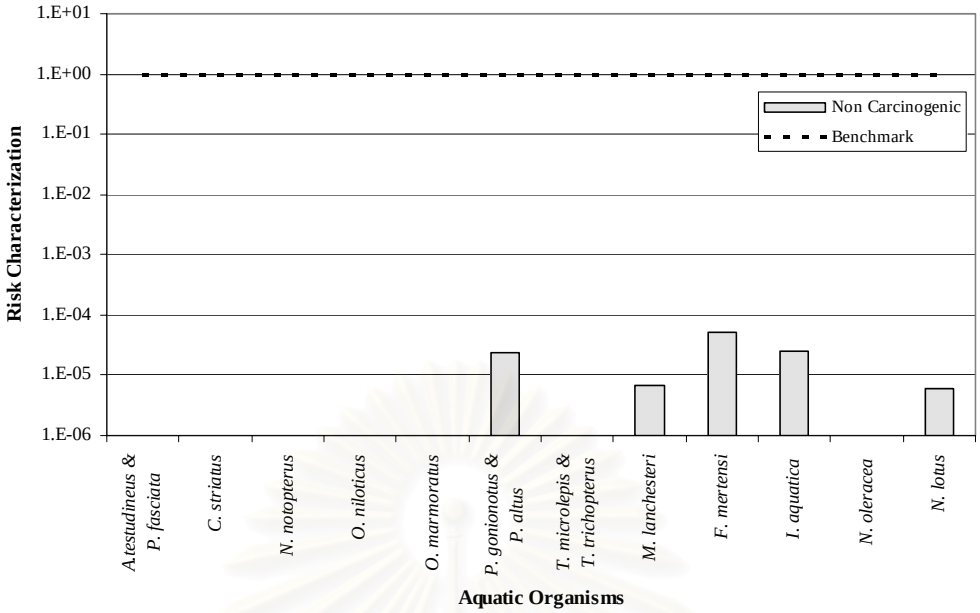


Figure 6.15 Non cancer hazardous ratios of methoxychlor for daily aquatic organisms' consumption by local population in Khlong 7, Rangsit agricultural area, central Thailand

6.4 Conclusions

There are a number of important limitations in this study. For example, this investigation did not consider (1) potentially different risks to separate age groups; (2) risks to populations residing in areas close to Rangsit agricultural area; (3) risks from other routes i.e. dermal and inhalation contact; and (4) risks from cooked aquatic organisms retained from Khlong 7. Research along this line should be available in the future. Overall, despite the limitations associated with the analysis, the assessment undertaken indicates a potential cancer risk due to OCPRs contamination in aquatic organisms. This presents an important step toward a more comprehensive understanding and organochlorine exposures via aquatic organism consumption in Khlong 7, Rangsit agricultural area. With an established dietary database for the local population of Khlong 7, more comprehensive risk assessments can be conducted when other contaminants such as PCBs, other pesticide groups, etc. are present.

CHAPTER VII

RISK MANAGEMENT OF ORGANOCHLORINE PESTICIDE RESIDUES, A CASE STUDY: RANGSIT AGRICULTURAL AREA, CENTRAL THAILAND

7.1 Introduction

Over a hundred years ago, King Rama the V of Siam, previous name of Thailand, initiated the Rangsit irrigation system project in the 1880s. About 20 straight canals of 20-30 km lengths running from south to north at about 2km intervals at the central part of Thailand was excavated. Currently, this man-made irrigation-network-system consists of 14 sub-canals in the Rangsit agricultural area in great plain of the Chao Phraya River, Pathum Thani province. This area is divided into two parts the upper and lower part by the Rangsit-Prayulasakdi canal. The edges of the Rangsit agricultural area are bordered by Bangkok to the south, Saraburi province to the north, Nakornayok province to the east, and the western edge is positioned in the Pathum Thani province. This area covers Thanyaburi, Klong luang, and Nong sua districts in Pathum Thani province (National science museum, 2002).

In order to increase the rice-growing areas for more export of rice production; this area has been intentionally designated as the main agricultural area of the country. Thailand's agricultural sector had shifted from labor- to machine- intensive farming practices since the 1960s. The importation of pesticides rose remarkably from approximately 2,000 tons of active ingredients in 1957 to approximately 4,000 tons in 1962 and then 37,039 tons in 2001. In the early phases of import the country was largely limited to types classified as organochlorine, organophosphate, and carbamate. Eventually new pesticide and other types of synthetic substances such as pyrethroids and extracts from plants and microorganisms were introduced in Thailand (NIP/POPs Coordination, 2005). Consequently, pesticides may reach the soil through direct application to the soil surface, incorporation in the top few inches of soil, or during application to crops. They can also enter ground water resources and surface run-off during rainfall, thereby contributing to the risk of human health and environmental contamination.

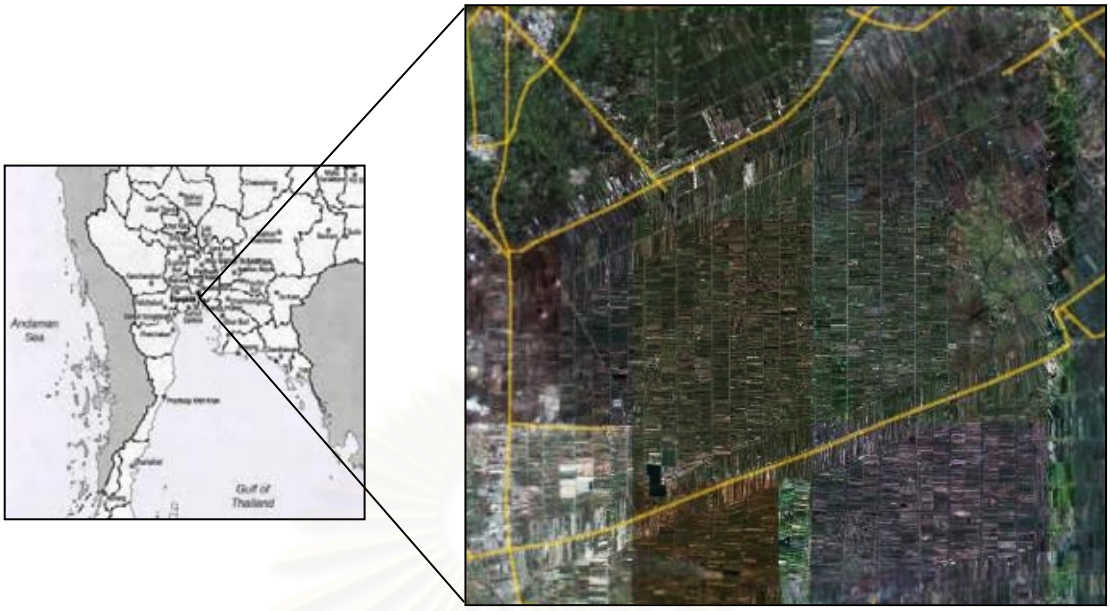


Figure 7.1 Arial photograph of Rangsit agricultural area, Central Thailand (source: http://earth.google.com/)

7.2 Use of Organochlorine Pesticides (OCPs) in the Past

Since 1974, OCPs have been imported to Thailand and widely used in agriculture and for public health purposes (see Appendix E). NIP/POPs Coordination (2005) wrote about the usage of some OCPs in Thailand and showed that: (1) aldrin was an insecticide applied to soil for termite control and other soil pests such as corn rootworm. The chemical was also used to control insects in grain storage and ectoparasites on cattle. (2) chlordane was used extensively for termite and ant control in buildings, nurseries and forest plantations. Chlordane was a broad-spectrum insecticide used to control pests on a wide range of crops. (3) dieldrin as a very effective pesticide applied against termite in buildings, crops, nurseries and forest plantations. The main use in the past also focused on locust control as well as grain storage and ectoparasites on cattle. (4) DDT was used widely to control of malaria, typhus, and other diseases spread by insects. It was also applied widely on crops and soil to protect against insect pests, e.g. Lepidoptera. (5) endrin was used on several crops such as rice, maize, cotton, and sugarcane to control for Lepdoptera. It is also used to control rodents in houses and stores. And (6) heptachlor was used to kill soil insects and termites. The usage was also extended to crop pests, grasshoppers and mosquitoes.

7.3 Organochlorine Pesticide Residues in Rangsit Agricultural Area

To prevent harm that may be inflicted upon humans, animals, plants, and the environment, all OCPs were banned in Thailand from 1981 to 2004 (see Appendix E). However, according to Anat and Paul (2000), OCP residues were still persistent in environment for long periods of time, especially organochlorine and organophosphate residues, which have been found in soil, water, and agricultural products throughout the country. As mentioned in other reported data, low concentrations of organochlorine residues have been found in sediment, water, fish, invertebrates, and aquatic plants of Khlong (canal) 7, Rangsit agricultural area (Thongkongowm *et al.*, 2006; Rohitrattana *et al.*, 2006; Siriwong *et al.*, 2006; Siriwong *et al.*, 2007). Furthermore, Thirakhupt (2006b) indicated that organochlorine pesticide residues (OCPRs) found in both Khlong 7 and Raphi Phat canal (on the upstream side of each sub canals) were similar. Raphi Phat canal supplies water to each sub canal, which may be causing the distribution of OCPRs in this area to be similar.

7.4 Risk Management

"Risk" is best described as "the chance of loss (or gain)." The management of risk builds on the result of risk assessment. The outcomes of all risk management actions should be the reduction of risk (Ritter *et al.*, 1995b). According to Chapter VI, the results indicated that the local population in Khlong 7 communities, Rangsit agricultural area may have a lifetime risk potential for cancer due to 9 contaminants: α-, β-HCH, heptachlor, heptachlor epoxide, aldrin, dieldrin, 4,4'-DDE, 4,4'-DDD, and 4,4'-DDT. These contaminates have been found in edible aquatic organisms such as fish, Lanchester's freshwater prawn (*Macrobrachium lanchesteri*), freshwater snail (*Filopaludina mertensi*), vegetables such as swamp morning-glory (*Ipomomea aquatica*), neptunia (*Neptunia oleracea*), and water lily (*Nymphaea lotus*). Due to the ban of OCPRs and the release of residues from the non point sources, it can be difficult to set implementation guidelines to reduce these residues. Therefore, the appropriate risk management recommendations to help local communities avoid and protect themselves from OCPs are listed in the following sections.

7.4.1 Community concern and risk communication

7.4.1.1 Communities awareness

Local government should provide the risk assessment information and communication to local communities. It is possible to build up the announcement signboard along the canal. The aim is to inform people who consume aquatic organisms from the canal about the risk of OCPRs and how to minimize the hazard.

According to the removal mechanisms of OCPRs in aquatic plant, there is the potential for using phytoremediation (Nzengung and Jeffers, 2001). Many local species found along Khlong 7 were thus recommended in Siriwong *et al.*, (2007). For example, alligator weed (*Alternanthera philoxeroides*) and climbing dayflower (*Commelina diffusa*) should be promoted to grow along the bank of the canal to absorb and breakdown the residues of contaminants from soil and run off of OCPRs that have high bioaccumulation factor (BAF) values (Siriwong *et al.*, 2007). Water hyacinth (*Eichhornia crassipes*) should also be systematically planted in the canals to remove OCPRs with high bioconcentration factor (BCF) values (Siriwong *et al.*, 2007).

Furthermore, excavation of canals should be avoided since OCPRs in sediment may be released from sediment particle to water body causing accumulation of contaminants by aquatic organisms.

7.4.1.2 Individual household awareness

Residue levels in prepared food often get reduced substantially when the raw commodity is subjected to trimming, washing, and cooking. Several generalizations about specific food preparation and cooking techniques reported by US EPA (200b) that trimming fish is an important consideration in reducing the OCPs ingested by consumers. For example, raw skin-off fillets had an average of 50 percent of the residues found in raw skin-on fillets. The skin-off fillets had both the belly flap and the lateral line and its associated fat trimmed off, while the skin-on fillet had only the belly flap removed. Cooking methods that allow the separation of the cooked muscle from the skin (pan frying, poaching, broiling, baking) reduce the amount of chemical

contaminants the consumer would ingest over such cooking methods as deep frying where both the skin and cooked muscle are consumed together. As a cooking process, smoking resulted in significantly greater reductions (40 to >50 percent) of OCPs (DDT,DDE, DDD, chlordane complex, HCH, dieldrin, heptachlor epoxide, toxaphene) than other cooking methods tested such as baking, charbroiling, salt boiling, deep fat frying, and canning.

Therefore, household should wash raw commodities thoroughly before cooking and/or consuming. Cooking with heat is also recommended to reduce the residues of OCPs. Changing eating behavior can reduce the risk of cancer from consumption of aquatic organisms contaminated by OCPs. When consuming aquatic organisms it is also better to eat a variety of aquatic species. Furthermore, adults should generally be checked for cancer at least once a year.

7.4.2 National government agencies concern

During 1970-1990, government research institutes had set up programs to monitor the impact of OCPRs on the environment and human health. The results of the investigations showed no alarming situation (NIP/POPs Coordination, 2005). On the other hand, the cancer risk from consumption of OCPRs to local populations of Rangsit agricultural area still persists (reported in Chapter VI). For the long-term, this study recommends that the national government periodically monitor water, sediment, and organisms of the Rangsit agricultural area for OCPRs. When there is no longer a risk present, the above monitoring program can be ignored. Moreover, the risk information and prevention activities should be addressed to the Rangsit agricultural community.

CHAPTER VIII

CONCLUSIONS

The entire study was conducted at Khlong 7 Rangsit agricultural area from 2003 to 2007. Focusing on organochlorines, banned pesticides, this work consisted of 2 main parts: (1) Organochlorine pesticide residues in aquatic ecosystem and (2) Health risk assessment of local agricultural community.

In the first part, it reveals that the presences of organochlorine residues were still existed in the freshwater ecosystem of Khlong 7 such as water, sediment, aquatic plants, plankton, vertebrates (fish), and invertebrates. Indeed, even though organochlorines were banned; residues are still circulated and magnified through the food web from the lowest up to the highest trophic levels. Moreover, the comprehensive food web of the freshwater ecosystem including bioconcentration, bioaccumulation, and biomagnification of organochlorine pesticide residues were accomplished in this part.

In the second part, the risk assessment shows that local population in Khlong 7 may confront the lifetime risk potential for cancer associated with the consumption of some aquatic organisms contaminated with organochlorine residues. Consequently, appropriated risk management had been recommended aimed to reduce the risk of this community. Furthermore, an established dietary database for the local populations of Khlong 7 was completed. It can be used for other risk assessments with different contaminants in future works.

According to both parts, organochlorine pesticide residues management framework was summarized in figure 8.1. Particularly, this diagram can be applied in future works for other areas and other banned hazardous chemicals to achieve the risk reduction.

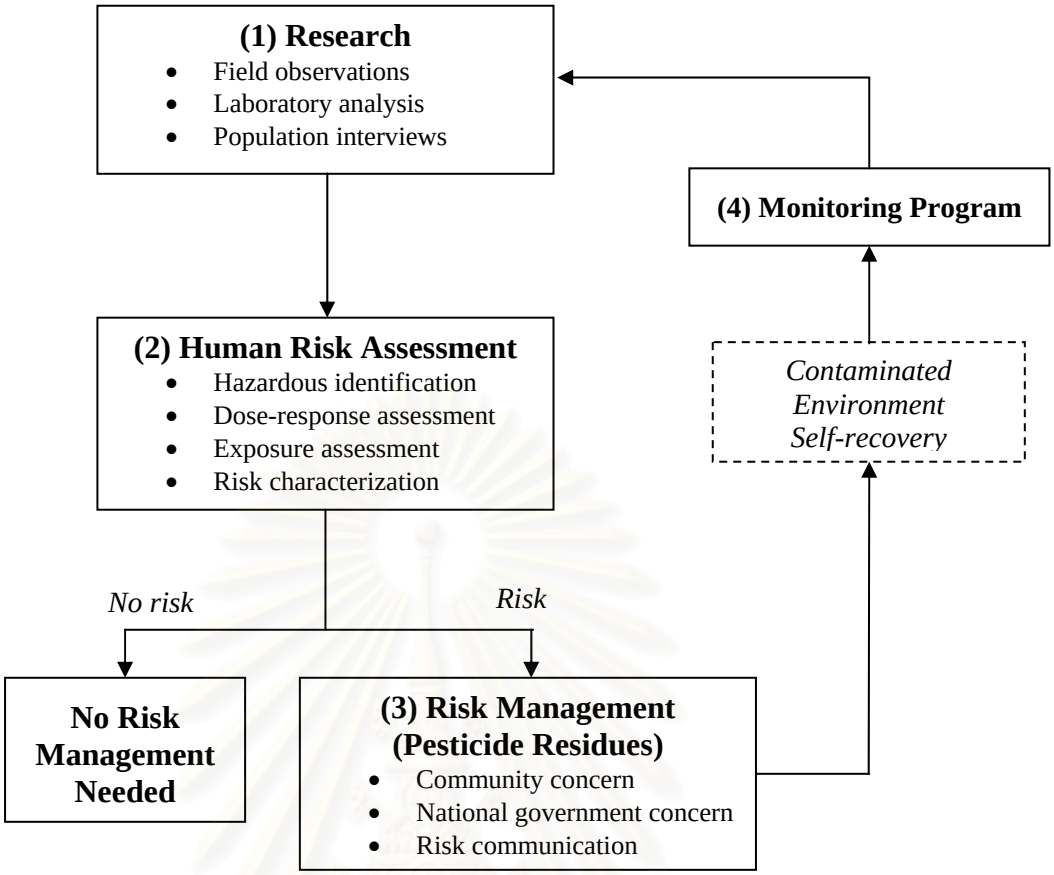


Figure 8.1 Organochlorine pesticide residues management framework

The first of the 4 stages is a phase of research involving with the collection of data from field observation, laboratory analysis, and population interviews. The results of the research stage are linked to human health risk assessment stage, which is, consisting of 4 steps: (1) hazardous identification, (2) dose-response assessment, (3) exposure assessment, and (4) risk characterization. When a risk is presented, risk management stage is needed. The risk manager, who can be a regulatory authority, or a unit responsible for polluting, or the victim of pollution, should address the risk information and prevention activities to the community. For the long-term, the stage of monitoring program is recommended. The loop of OCPRs management should be a continuous and stepwise process until there is no longer a risk present.

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APPENDICES

APPENDIX A

THE SUMMARY OF ORGANOCHLORINE PESTICDE RESIDUES IN THE ENVIRONMENTAL COMPARTMENTS OF KHLONG 7, RANGSIT AGRICULTURAL AREA, CENTRAL THAILAND

Table 1-A The concentration of OCPRs in environmental compartments (means ± S.E.) (ppb) of Khlong 7, Rangsit agricultural area, Pathum Thani Province, Thailand from June 2004 to May 2007

Environment Compartments (units)	**n**	**The concentration of OCPRs (mean ± S.E.) (ppb)**						
		∑ HCH[a]	**Heptachlor & Heptachlor epoxide**	**Aldrin & Dieldrin**	**DDT & derivatives**	**∑ Endosulfan**[b]	**Endrin & Endrin aldehyde**	**Methoxychlor**
Water (ng/mL)	108	0.01 ± 0.002	0.007 ± 0.001	0.007 ± 0.001	0.02 ± 0.001	0.08 ± 0.01	0.005 ± 0.001	0.001 ± 0.003
Sediment (ng/g dry wt.)	108	9.36 ± 0.27	14.67 ± 0.48	2.97 ± 0.23	12.05 ± 0.30	6.36 ± 0.25	0.78 ± 0.12	0.04 ± 0.02
Plankton (ng/g wet wt.)								
Phyto- and zoo- plankton	51	1.80 ± 0.34	1.78 ± 0.47	1.10 ± 0.15	3.65 ± 0.58	3.29 ± 0.28	0.69 ± 0.18	0.10 ± 0.03
Aquatic plants (macrophyton) (ng/g wet wt.)								
Alternanthera philoxeroides	30	8.51 ± 0.53	6.00 ± 0.34	2.42 ± 0.30	7.99 ± 1.16	13.35 ± 0.85	0.15 ± 0.09	0.26 ± 0.09
Commelina benghalesis	12	6.33 ± 0.59	5.80 ± 0.23	0.72 ± 0.11	8.17 ± 1.42	9.53 ± 1.22	0.14 ± 0.09	0.13 ± 0.07
Eichhornia crassipes	84	5.44 ± 0.32	5.90 ± 0.21	2.13 ± 0.19	9.25 ± 0.55	7.91 ± 0.49	1.49 ± 0.26	0.79 ± 0.08
Ipomoea aquatica	42	7.59 ± 0.64	5.39 ± 0.27	2.88 ± 0.74	6.94 ± 0.60	13.79 ± 1.47	0.76 ± 0.21	0.34 ± 0.11
Ludwigia adscendens	9	9.28 ± 0.93	4.87 ± 1.00	3.22 ± 0.74	9.12 ± 0.60	9.99 ± 1.01	5.34 ± 1.34	0.55 ± 0.14
Neptunia oleracea	6	36.49 ± 6.79	4.19 ± 1.16	2.90 ± 0.23	19.61 ± 2.38	14.03 ± 0.29	< 0.002	< 0.005
Nymphaea lotus	57	12.76 ± 1.36	5.64 ± 0.32	2.14 ± 0.25	8.86 ± 1.32	8.22 ± 0.86	0.73 ± 0.21	0.09 ± 0.02
Pistia stratiotes	33	10.59 ± 1.11	5.48 ± 0.30	2.53 ± 0.41	11.12 ± 1.91	13.01 ± 1.70	2.14 ± 0.50	0.10 ± 0.04
total	273	9.11 ± 0.48	5.65 ± 0.12	2.32 ± 0.15	9.08 ± 0.45	10.37 ± 0.43	1.19 ± 0.13	0.38 ± 0.04

Environment Compartments (units)	n	The concentration of OCPRs (mean ± S.E.) (ppb)						
		∑ HCH[a]	Heptachlor & Heptachlor epoxide	Aldrin & Dieldrin	DDT & derivatives	∑ Endosulfan[b]	Endrin & Endrin aldehyde	Methoxychlor
				Invertebrates[c] (ng/g wet wt.)				
Filopaludina mertensi	57	42.27 ± 4.59	18.92 ± 2.30	18.33 ± 1.58	79.62 ± 8.82	27.87 ± 3.44	9.34 ± 1.80	1.35 ± 0.27
Macrobrachium lanchesteri	93	27.08 ± 2.13	14.52 ± 0.85	5.88 ± 0.46	53.04 ± 7.85	36.69 ± 5.70	5.82 ± 1.23	0.52 ± 0.15
Pomacea sp.	72	34.34 ± 2.64	19.02 ± 1.54	15.70 ± 1.73	47.83 ± 5.07	36.51 ± 3.97	16.76 ± 3.15	1.69 ± 0.27
total	222	33.34 ± 1.75	17.11 ± 0.86	12.26 ± 0.80	58.17 ± 4.38	34.37 ± 2.86	10.30 ± 1.27	1.11 ± 0.13
				Vertebrates (fish)[d] (ng/g wet wt.)				
Anabas testudineus	6	2.13 ± 0.53	3.52 ± 1.26	1.10 ± 0.23	5.71 ± 1.40	10.64 ± 4.21	0.84 ± 0.28	< 0.005
Channa striatus	9	20.95 ± 0.51	28.64 ± 0.50	22.45 ± 0.15	57.66 ± 1.08	46.22 ± 0.67	< 0.002	< 0.005
Clupeichthys aesarnensis	15	0.88 ± 0.27	1.78 ± 1.00	0.39 ± 0.15	2.54 ± 0.68	0.56 ± 0.19	0.13 ± 0.07	< 0.005
Cyclocheilichthys amatus	15	2.26 ± 0.32	1.58 ± 0.32	0.10 ± 0.07	3.92 ± 1.95	0.67 ± 0.29	0.26 ± 0.14	0.02 ± 0.02
Dangila spilopleura	9	2.36 ± 0.56	2.75 ± 0.16	0.85 ± 0.30	8.20 ± 2.52	1.76 ± 0.37	< 0.002	< 0.005
Esomus metallicus	24	3.30 ± 1.02	2.78 ± 0.63	2.05 ± 0.82	3.37 ± 0.53	2.66 ± 0.74	1.02 ± 0.47	0.01 ± 0.01
Henicorhynchus siamensis	3	< 0.05	2.45 ± 0.19	< 0.05	3.74 ± 0.29	0.63 ± 0.63	0.00 ± 0.00	< 0.005
Heterobagrus bocouri	3	0.06 ± 0.01	0.97 ± 0.13	0.51 ± 0.12	14.30 ± 0.07	39.52 ± 0.48	1.69 ± 0.05	< 0.005
Hypsibarbus wetmorei	3	1.12 ± 0.04	0.35 ± 0.04	< 0.05	5.36 ± 0.08	0.44 ± 0.15	< 0.002	< 0.005
Macrognathus siamensis	9	5.09 ± 0.53	4.46 ± 0.73	6.83 ± 1.31	44.76 ± 8.06	61.23 ± 1.36	8.77 ± 1.48	0.58 ± 0.24
Micronema bleekeri	3	0.69 ± 0.35	0.13 ± 0.07	0.96 ± 0.26	8.37 ± 0.23	9.61 ± 0.81	2.42 ± 1.15	< 0.005
Mystus cavasius	6	0.68 ± 0.07	0.42 ± 0.05	1.18 ± 0.15	14.62 ± 0.36	35.14 ± 2.50	1.59 ± 0.10	< 0.005
Mystus mysticetus	6	1.13 ± 0.33	0.76 ± 0.06	0.65 ± 0.15	6.57 ± 0.85	31.46 ± 0.85	0.51 ± 0.28	< 0.005
Notopterus notopterus	15	0.96 ± 0.16	1.14 ± 0.39	0.86 ± 0.14	5.19 ± 0.54	6.97 ± 1.02	0.37 ± 0.11	< 0.005
Oreochromis niloticus	3	8.37 ± 0.25	6.16 ± 0.07	2.03 ± 0.13	15.41 ± 0.11	17.71 ± 0.13	3.83 ± 0.09	< 0.005
Oxyeleotris marmoratus	15	9.87 ± 1.17	21.32 ± 2.74	15.92 ± 1.93	25.71 ± 2.85	32.72 ± 3.82	0.27 ± 0.27	< 0.005
Oxygaster anomalura	3	3.12 ± 0.08	3.50 ± 0.14	0.82 ± 0.11	5.87 ± 0.24	7.06 ± 0.08	0.58 ± 0.04	< 0.005
Ozyrias minutillus	3	0.57 ± 0.04	3.95 ± 0.26	0.57 ±0.02	6.77 ± 0.70	1.89 ± 0.10	< 0.002	< 0.005
Pangasius sutchi	3	0.69 ± 0.07	3.12 ± 0.14	1.01 ± 0.20	16.44 ± 0.21	2.87 ± 0.12	3.02 ± 0.12	< 0.005
Parachela siamensis	3	3.06 ± 0.13	0.41 ± 0.07	< 0.05	3.30 ± 1.05	0.87 ± 0.06	< 0.002	< 0.005

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Environment Compartments (units)	n	The concentration of OCPRs (mean ± S.E.) (ppb)						
		∑ HCH[a]	Heptachlor & Heptachlor epoxide	Aldrin & Dieldrin	DDT & derivatives	∑ Endosulfan[b]	Endrin & Endrin aldehyde	Methoxychlor
Paralaubaca harmandi	6	3.62 ± 0.58	3.01 ± 0.51	0.69 ± 0.15	4.73 ± 0.43	6.64 ± 0.92	0.56 ± 0.21	< 0.005
Parambassis siamensis	15	11.86 ± 0.14	27.91 ± 0.64	19.34 ± 0.20	23.73 ± 0.34	40.02 ± 0.52	0.08 ± 0.05	< 0.005
Parambassis wolffi	6	5.17 ± 0.11	5.06 ± 0.16	3.44 ± 0.11	8.25 ± 0.09	10.64 ± 0.18	4.47 ± 0.22	< 0.005
Pristolepis fasciatus	3	5.85 ± 0.11	8.96 ± 0.06	7.51 ± 0.21	14.39 ± 0.12	33.19 ± 0.08	2.31 ± 0.05	< 0.005
Probarbus labeamajor	15	2.02 ± 0.25	< 0.04	< 0.05	0.85 ± 0.46	0.46 ± 0.15	< 0.002	< 0.005
Probarbus labeaminor	6	1.59 ± 0.12	0.67 ± 0.04	< 0.05	5.13 ± 2.29	9.98 ± 4.13	1.91 ±0.85	< 0.005
Puntioplites proctozysron	18	4.87 ± 0.91	1.79 ± 0.36	1.69 ± 0.31	8.28 ± 0.56	8.52 ± 1.99	0.56 ± 0.18	< 0.005
Puntius altus	3	1.20 ± 0.02	1.77 ± 0.08	0.83 ± 0.04	7.30 ± 0.05	3.21 ± 0.09	7.10 ± 0.18	< 0.005
Puntius brevis	6	0.38 ± 0.31	2.61 ± 0.33	0.94 ± 0.33	14.15 ± 1.44	2.00 ± 0.87	< 0.002	< 0.005
Puntius gonionotus	30	2.13 ± 0.38	3.35 ± 0.39	0.73 ± 0.22	4.16 ± 0.62	3.18 ± 0.49	0.60 ± 0.28	0.04 ± 0.04
Puntius schwanenfeldi	12	5.35 ± 1.47	4.15 ± 0.56	2.40 ± 0.42	10.83 ± 3.16	3.84 ± 0.46	< 0.002	< 0.005
Rasbora borapetensis	15	1.74 ± 0.34	2.52 ± 0.50	0.53 ± 0.20	4.40 ± 0.86	4.01 ± 1.01	0.22 ± 0.12	< 0.005
Rasbora sp.1	9	6.58 ± 1.47	1.52 ± 0.90	2.26 ± 0.98	5.45 ± 1.12	8.27 ± 2.74	0.27 ± 0.27	< 0.005
Rasbora tornieri	6	0.94 ± 0.21	0.35 ± 0.22	0.92 ± 0.40	1.29 ± 0.45	1.80 ± 0.59	0.61 ± 0.22	< 0.005
Toxotes chatareus	3	< 0.05	2.44 ± 0.14	1.42 ± 0.52	6.68 ± 0.44	2.65 ± 0.26	0.96 ± 0.29	< 0.005
Trichogaster microlepis	24	4.08 ± 0.50	4.32 ± 0.52	2.18 ± 0.43	23.75 ± 2.92	7.80 ± 0.79	3.37 ± 0.53	< 0.005
Trichogaster trichopterus	24	3.71 ± 0.56	4.88 ± 0.43	3.17 ± 0.65	12.66 ± 1.41	11.90 ± 1.62	3.59 ± 0.69	< 0.005
Trichopsis pumila	12	1.68 ± 0.30	2.41 ± 0.32	1.11 ± 0.16	4.53 ± 0.44	1.79 ± 0.11	< 0.002	< 0.005
Trichopsis vittatus	27	2.54 ± 0.41	2.94 ± 0.59	1.70 ± 0.41	3.43 ± 0.86	7.20 ± 2.18	0.82 ± 0.21	0.01 ± 0.01
Xenantodon cancilla	24	2.79 ± 0.39	4.43 ± 0.49	1.78 ± 0.25	6.38 ± 0.92	9.04 ± 1.37	0.45 ± 0.17	< 0.005
Zenarchopterus ectuntio	6	2.01 ± 0.34	1.99 ± 1.02	0.20 ± 0.10	< 0.04	1.51 ± 0.45	0.79 ± 0.23	< 0.005
total	426	3.70 ± 0.21	4.88 ± 0.35	3.08 ± 0.27	10.29 ± 0.92	10.85 ± 0.72	1.14 ± 0.11	0.02 ± 0.01

[a] ∑ HCH, sum of α-, γ-, β- and δ-HCH
[b] ∑ Endosulfan, sum of α-, β-, and -sulfate endosulfan
[c] collaborated with Rohitrattana (2005).
[d] collaborated with Thongkongoum (2005).

APPENDIX B

THE CHROMATOGRAMS OF 17 ORGANOCHLORINE PESTICIDE RESIDUES IN DIFFERENT MATRICES

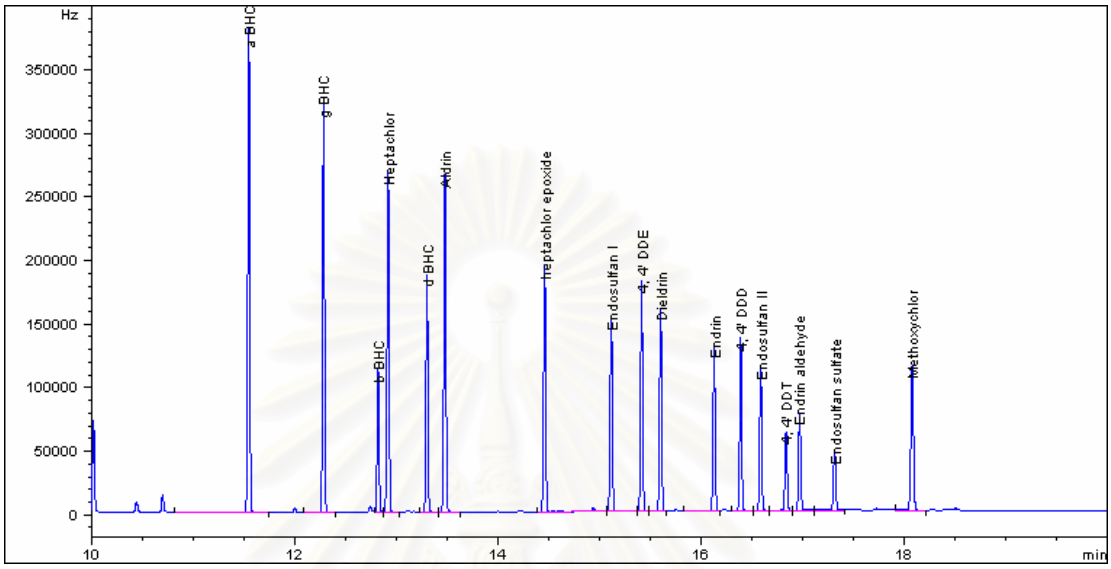


Figure 1-B The chromatogram of 17 mixed organochlorine pesticide standard 50 ng/mL using DB-35MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) coated with 35% diphenyl polysiloxane

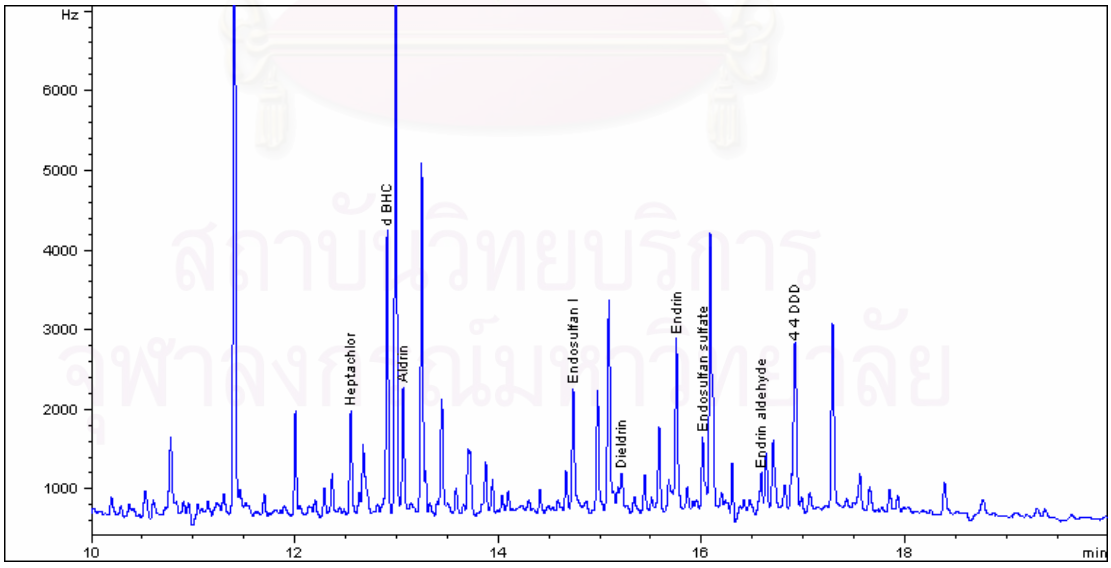


Figure 2-B The chromatogram of OCPRs in water sample using DB-35MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) coated with 35% diphenyl polysiloxane

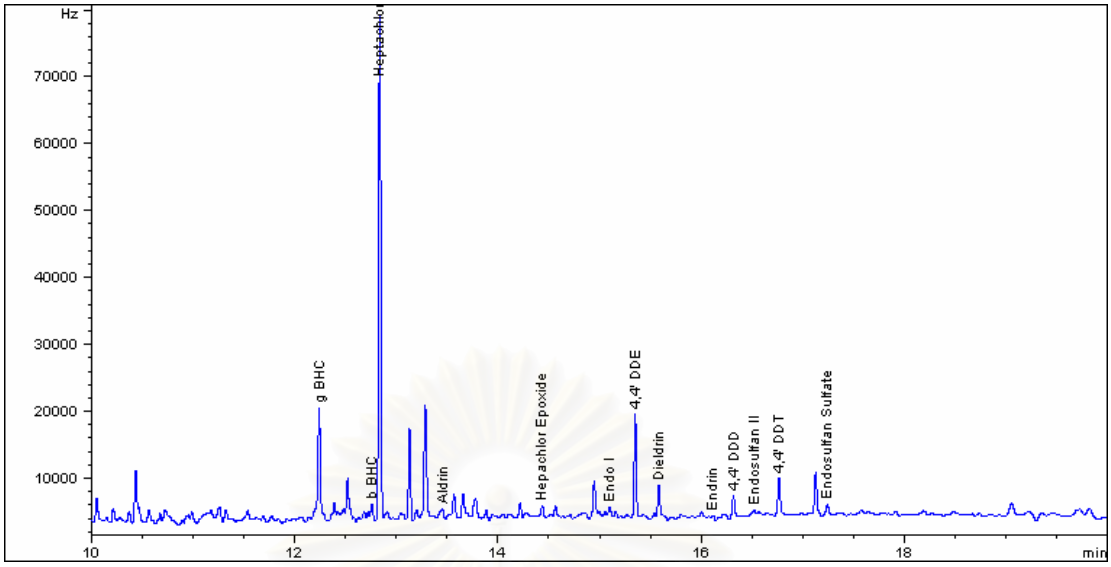


Figure 3-B The chromatogram of OCPRs in sediment sample using DB-35MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) coated with 35% diphenyl polysiloxane

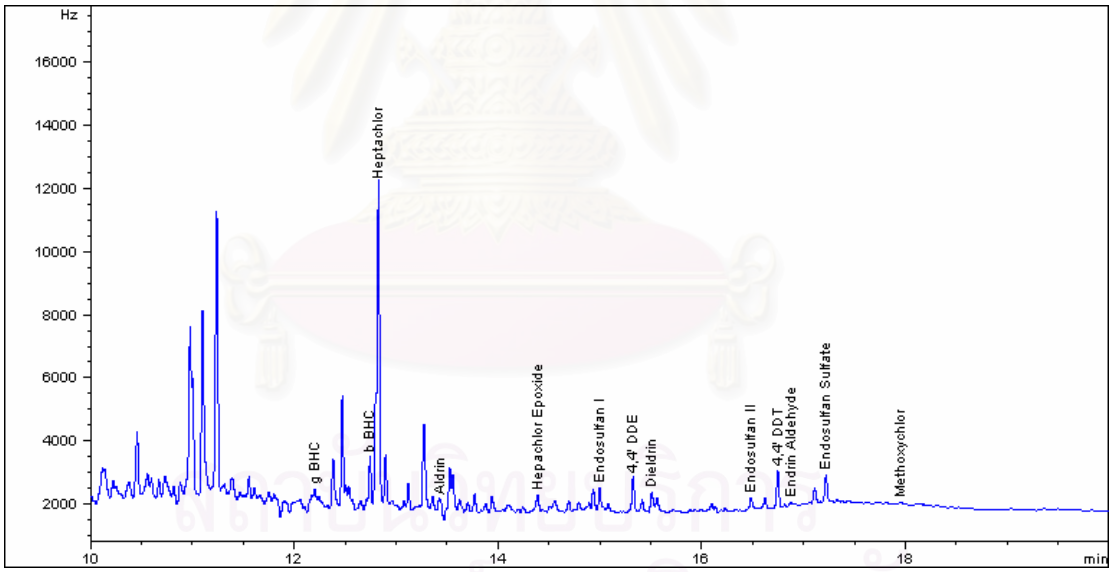


Figure 4-B The chromatogram of OCPRs in aquatic plant sample (*Eichhornia crassipes*) using DB-35MS fused silica capillary column (30 m length, 0.25 mm, i.d., 0.25 µm film thickness) coated with 35% diphenyl polysiloxane

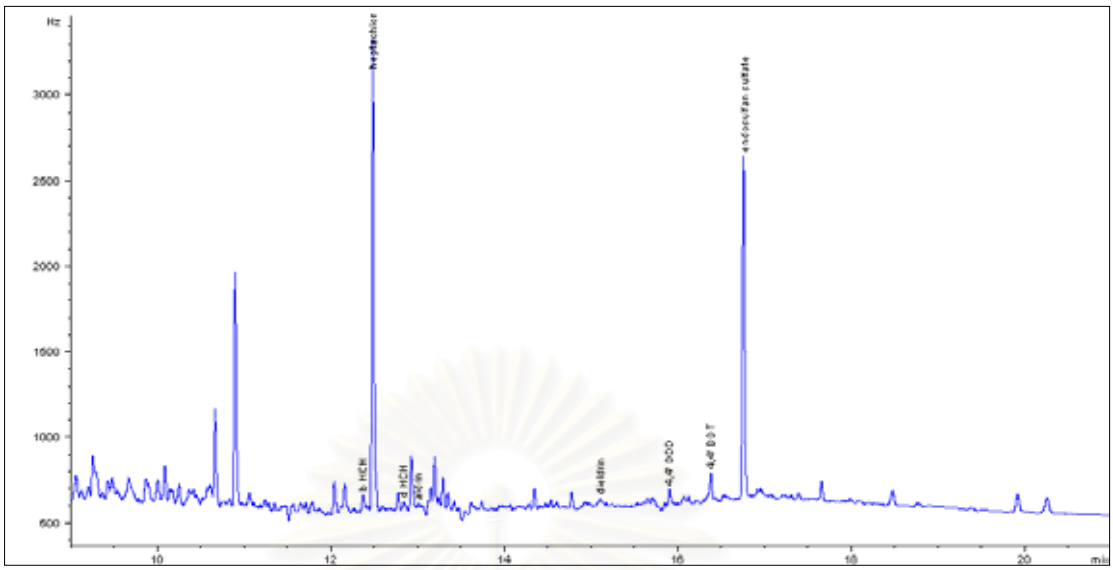


Figure 5-B The chromatogram of OCPRs in plankton sample using DB-35MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) coated with 35% diphenyl polysiloxane

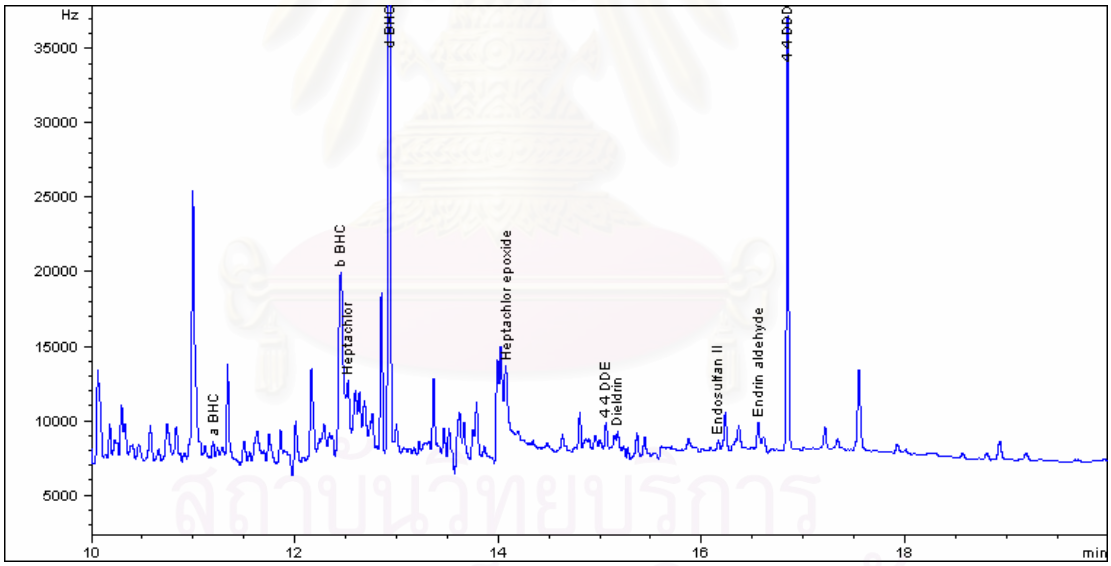


Figure 6-B The chromatogram of OCPRs in fish sample (*Channa striatus*) using DB-35MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) coated with 35% diphenyl polysiloxane

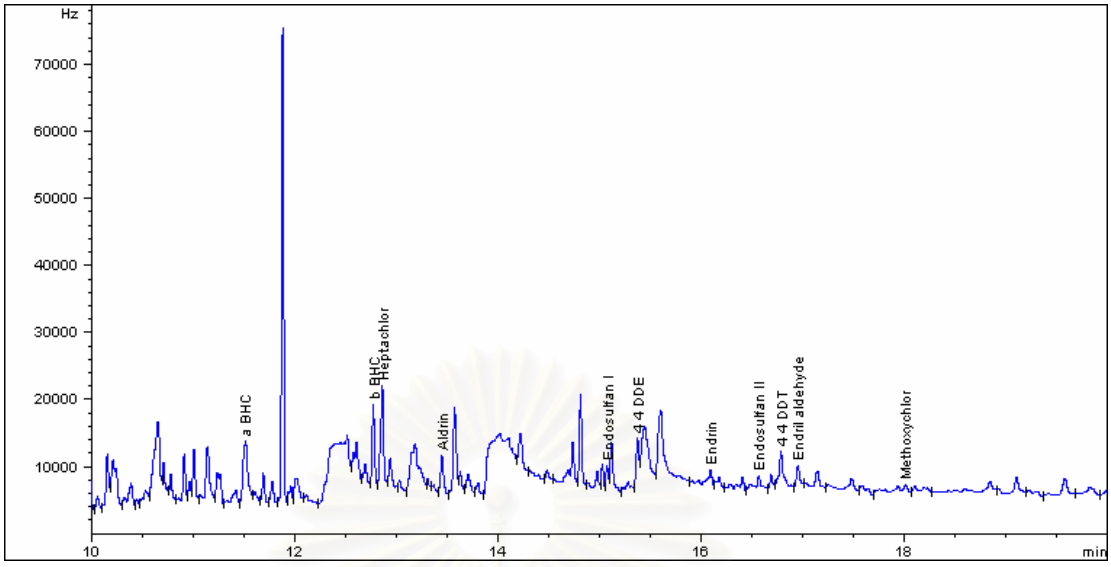


Figure 7-B The chromatogram of OCPRs in shrimp sample (*Macrobrachium lanchesteri*) using DB-35MS fused silica capillary column (30 m length, 0.25 mm, i.d., 0.25 µm film thickness) coated with 35% diphenyl polysiloxane

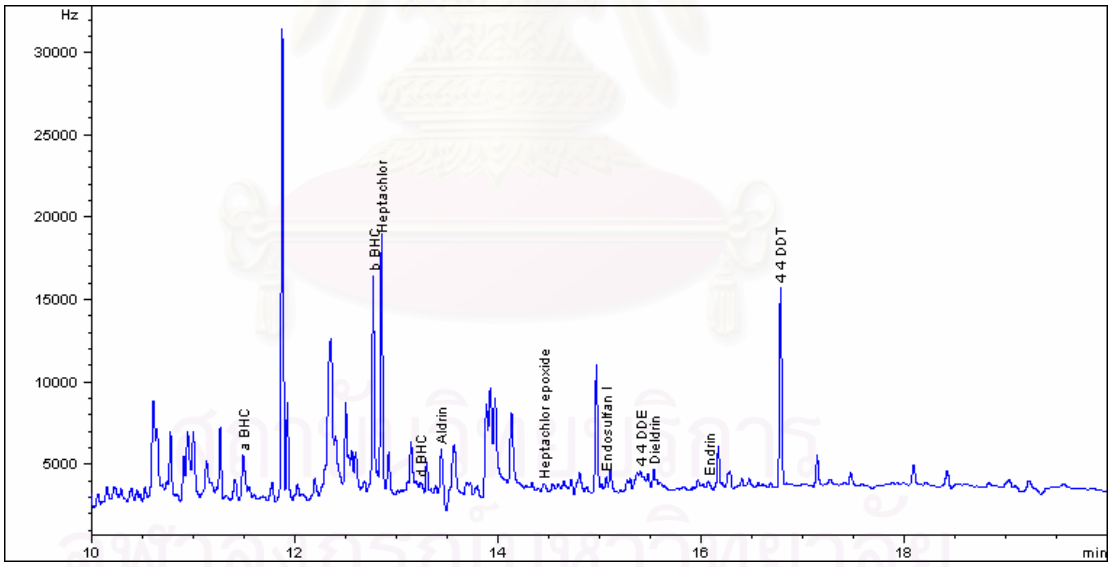


Figure 8-B The chromatogram of OCPRs in snail sample (*Pomacea* sp.) using DB-35MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) coated with 35% diphenyl polysiloxane

APPENDIX C

QUALITY CONTROL

Table 1-C The limit of detection (LOD), limit of quantitation (LOQ), and method detection limit (MDL) of 17 OCPRs in different matrices

Organochlorine Pesticides	LOD *(ng/mL)*	LOQ *(ng/mL)*	MDL (ppb)						
			Water ♠ *(ng/mL)*	***Sediment*** ♥ *(ng/g dry wt.)*	***Aquatic Plants*** ♥ *(ng/g wet wt.)*	***Plankton*** ♠ *(ng/g wet wt.)*	***Fish*** ♣ *(ng/g wet wt.)*	***Invertebrates*** ♥ *(ng/g wet wt.)*	
								Shrimp	***Snail***
α-BHC	0.030	0.090	0.005	1.11	3.05	0.11	0.70	0.55	0.22
γ-BHC	0.050	0.200	0.005	1.22	2.20	0.06	1.00	1.46	2.37
β-BHC	0.010	0.050	0.004	1.09	3.96	0.11	0.73	2.24	1.63
Heptachlor	0.001	0.002	0.001	0.67	2.37	0.23	1.09	2.24	1.80
δ-BHC	0.040	0.100	0.006	1.07	1.93	0.09	1.00	1.77	0.67
Aldrin	0.020	0.050	0.001	1.21	0.63	0.24	1.35	1.67	1.64
Heptachlor epoxide	0.020	0.070	0.003	1.11	0.86	0.15	2.22	2.17	0.16
Endosulfan I	0.003	0.009	0.004	0.86	1.94	0.20	1.20	1.57	3.89
4,4'-DDE	0.040	0.100	0.003	0.74	2.34	0.09	1.40	1.46	3.89
Dieldrin	0.030	0.100	0.002	1.09	2.04	0.16	1.49	2.36	0.46
Endrin	0.020	0.070	0.005	0.74	1.72	0.32	1.40	2.73	1.52
4,4'-DDD	0.003	0.010	0.003	0.66	1.93	0.19	1.16	2.73	0.64
Endosulfan II	0.003	0.009	0.003	0.75	2.32	0.02	0.96	2.37	1.06
4,4'-DDT	0.002	0.008	0.002	1.46	2.10	0.05	1.06	3.32	2.77
Endrin aldehyde	0.010	0.040	0.004	1.32	1.37	0.13	0.70	3.71	0.87
Endosulfan sulfate	0.002	0.007	0.010	1.38	1.78	0.28	0.77	3.82	5.17
Methoxychlor	0.005	0.015	0.003	1.36	1.90	0.49	1.85	2.08	3.18

♠ at 5 ng/mL of the analyte concentration , ♣ at 10 ng/mL of the analyte concentration, and ♥ at 50 ng/mL of the analyte concentration

Table 2-C The relative standard deviations (RSD) and recoveries of 17 OCPRs in different matrices

Organochlorine Pesticides	RSD (%)							Matrices Spiked Recovery (%)						
	Water ♠	*Sediment* ♥	*Aquatic Plants* ♥	*Plankton* ♠	*Fish* ♣	*Invertebrates* ♥		*Water* ♣	*Sediment* ♥	*Aquatic Plants* ♥	*Plankton* ♠	*Fish* ♣	*Invertebrates* ♥	
						Shrimp	*Snail*						*Shrimp*	*Snail*
α-BHC	11.07	3.63	12.21	0.87	2.75	1.74	0.72	95.29	74.87	78.59	99.70	130.57	73.87	72.62
γ-BHC	10.68	3.76	8.30	0.49	5.38	5.06	8.08	98.74	77.62	75.28	100.52	95.76	67.84	68.84
β-BHC	8.49	3.32	11.15	0.94	2.96	7.66	4.33	99.44	75.11	79.24	95.70	127.53	104.06	88.30
Heptachlor	8.76	1.89	5.94	1.79	4.86	7.15	5.97	114.32	84.08	75.19	100.27	115.48	83.22	70.82
δ-BHC	8.41	3.24	7.21	0.79	4.16	5.66	1.85	119.61	77.34	73.55	88.66	123.80	73.44	85.26
Aldrin	3.42	3.88	3.15	2.12	5.83	5.60	4.20	72.43	79.99	65.54	88.93	119.36	70.92	91.47
Heptachlor epoxide	6.02	3.34	3.65	1.40	10.16	5.80	0.53	87.22	83.02	74.19	83.86	112.30	91.59	70.21
Endosulfan I	7.99	2.53	7.31	1.79	5.98	4.80	10.55	95.54	90.03	78.69	86.69	103.54	76.85	82.46
4,4'-DDE	7.64	2.26	8.60	0.75	7.59	3.89	7.84	76.91	85.81	70.65	99.17	95.24	88.33	116.29
Dieldrin	4.61	3.15	7.55	1.38	7.60	4.05	1.03	88.85	89.30	68.88	88.55	101.19	99.78	103.90
Endrin	11.00	2.34	5.51	2.86	6.73	6.94	3.20	90.90	80.57	98.15	88.61	107.20	92.11	111.55
4,4'-DDD	4.22	1.87	7.38	1.67	7.25	3.74	1.42	116.17	86.15	74.58	91.21	82.03	101.48	106.05
Endosulfan II	6.65	2.24	8.16	0.20	5.82	5.08	2.13	96.29	84.46	79.87	90.49	85.33	109.12	116.60
4,4'-DDT	5.96	3.69	5.06	0.47	4.99	7.52	8.38	84.79	90.66	103.32	88.73	109.38	103.43	77.58
Endrin aldehyde	7.48	3.82	5.37	0.99	4.18	9.53	2.89	115.83	88.98	76.91	103.32	85.97	95.82	70.24
Endosulfan sulfate	11.86	2.99	5.64	2.15	3.17	9.87	12.78	117.00	89.02	82.66	100.46	124.57	90.81	94.85
Methoxychlor	8.21	2.93	4.15	3.72	8.08	5.53	10.45	71.30	92.67	115.57	102.54	117.53	115.14	72.61

♠ at5 ng/mL of the analyte concentration, ♣ at 10 ng/mL of the analyte concentration, and ♥ at 50 ng/mL of the analyte concentration

Method validation (US EPA, 2000a)

1. Limit of detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. In chromatography, the detection limit is the injected amount that results in a peak with a height at least twice or three times as high as the baseline noise level. The limit of quantitation (LOQ) is the minimum injected amount that gives precise measurements, in chromatography typically requiring peak heights 10 to 20 times higher than baseline noise. Therefore, The LOD and LOQ are defined as the peak height of analyte in standard solution that signaled significantly different from the peak height of noise. The LOD and LOQ can be calculated by the equation below,

$$\mathrm{LOD} = 3\frac{Signal}{Noise} \qquad \text{(Equation C1)}$$

$$\mathrm{LOQ} = 10\frac{Signal}{Noise} \qquad \text{(Equation C2)}$$

2. Method detection limit (MDL)

The minimum concentration of an analyte in a given matrix (such fish, aquatic plant, invertebrate, etc.) that can be measured and reported with 95 percent confidence that concentration is greater than zero. The MDL is determined by multiplying the appropriate (i.e., n-1 degree of freedom) one-sided 95 percent Student's t-statistic ($t_{0.95}$) by the standard deviation (SD) obtained from a minimum of seven replicate analyses of a spiked matrix sample containing analyte of interest at a concentration three to five times the estimated MDL.

$$\mathrm{MDL} = t_{0.95(n-1)} \times SD \qquad \text{(Equation C3)}$$

3. Assessment of method accuracy

The accuracy is calculated as percent recovery from the analysis of reference materials, or laboratory control samples, as follows: The recovery percentage can be calculated by the equation below,

$$\%\ \text{Recovery} = [(M_s - M_u)/T_s] \times 100 \qquad \text{(Equation C4)}$$

where

M_s = Measured concentration of target analyte in the spiked sample

M_u = Measured concentration of target analyte in the unspiked sample

T_s = "True" concentration of target analyte added to the spiked sample

The concentration should cover the range of concern and should particularly include one concentration close to the quantitation limit. The expected recovery depends on the sample matrix, the sample processing procedure and on the analyte concentration. The AOAC manual (Peer Verified Methods program, 1993) includes a table 3-C with estimated recovery data as a function analyte concentration.

Table 3-C AOAC recommendation for analyte recovery at different concentrations [a]

Active Ingredient (%)	Analyte ratio	Unit	Mean recovery (%)
100	1	100%	98-102
>=10	10^{-1}	10%	98-102
>=1	10^{-2}	1%	97-103
>=0.1	10^{-3}	0.10%	95-105
0.01	10^{-4}	100 ppm	90-107
0.001	10^{-5}	10 ppm	80-110
0.0001	10^{-6}	1 ppm	80-110
0.00001	10^{-7}	100 ppb	80-110
0.000001	10^{-8}	10 ppb	60-115
0.0000001	10^{-9}	1 ppb	40-120

Source: [a] AOAC Peer Verified methods Program, (1993).

4. Assessment of method precision

The most commonly used estimation of precision is the relative standard deviation (RSD) or coefficient of variation (CV) for multiple samples. The % RSD was calculated from the equation as below;

$$\%\,\mathrm{RSD} = 100\frac{SD}{Mean} \qquad \text{(Equation C5)}$$

The acceptance criteria for precision depend very much on the type of analysis. While for compound analysis in pharmaceutical quality control precision of better than 1 % RSD is easily achieved, for biological samples the precision is more like 15% at the concentration limits and 10% at other concentration levels. For environmental and food samples, the precision is very much dependent on the sample matrix, the concentration of the analyte and on the analysis technique. It can vary between 2% and more than 20% (AOAC Peer Verified methods Program, 1993).

Table 4-C AOAC recommendation for analyte concentration versus precision (relative standard deviation, RSD) within or between day [a]

Analyte (%)	Analyte ratio	Unit	RSD(%)
100	1	100%	1.3
10	10^{-1}	10%	2.8
1	10^{-2}	1%	2.7
0.1	10^{-3}	0.10%	3.7
0.01	10^{-4}	100 ppm	5.3
0.001	10^{-5}	10 ppm	7.3
0.0001	10^{-6}	1 ppm	11
0.00001	10^{-7}	100 ppb	15
0.000001	10^{-8}	10 ppb	21
0.0000001	10^{-9}	1 ppb	30

Source: [a] AOAC Peer Verified methods Program, (1993).

APPENDIX D

ORGANOCHLORINE PESTICIDES PROPERTIES

Table 1-D HCH or BHC properties

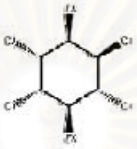
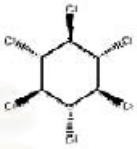
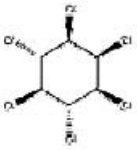
Characteristic	γ-BHC	α-BHC	β-BHC	δ-BHC
Synonym(s)	Gamma-benzenehexachloride; gamma-hexachlorocyclohexane; lindane	Alpha-benzenehexachloride; alpha-1,2,3,4,5,6-hexachlo- rocyclohexane; alpha-hexachlorane	Beta-benzenehexachloride; beta-hexachloro-benzene; beta-1,2,3, 4,5,6-hexachlorocyclohexane	Delta-benzenehexachloride; delta-1,2,3,4,5,6-hexachlorocyclohexane; delta-lindane
Chemical formula	$C_6H_6Cl_6$	$C_6H_6Cl_6$	$C_6H_6Cl_6$	$C_6H_6Cl_6$
Chemical structure				
Melting point	112.5 °C	159-160 °C	314-315 °C	141-142 °C
Boiling point	323.4 °C at 760 mmHg	288 °C at 760 mmHg	60 °C at 0.5 mmHg	60 °C at 0.36 mmHg
Water solubility	17 ppm; insoluble in water	10 ppm; 69.5 ppm at 28°C	5 ppm	10 ppm
Partition coefficients:				
Log K_{ow}	3.72	3.8	3.78	4.14
Log K_{oc}	3.0, 3.57	3.57	3.57	3.8
Vapor pressure	4.2×10^{-5} mmHg at 20 °C	4.5×10^{-5} mmHg at 25 °C	3.6×10^{-7} mmHg at 20 °C	3.5×10^{-5} mmHg at 25 °C

Table 2-D DDT and derivatives properties

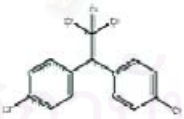
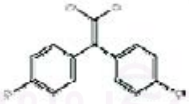
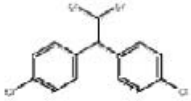
Characteristic	4,4′-DDT	4,4′-DDE	4,4′-DDD
Synonym(s)	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; dichlorodiphenyltrichloro ethane; DDT; 1,1′-(2,2,2-trichloro ethylidene)bis(4-chloro-benzene)	1,1-dichloro-2,2-bis(*p*-chlorophentl) ethylene; dichlorodiphenyldichloro ethane;1,1′-(2,2-dichloroethylidene) bis(4-chlorobenzene)	1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane; 1,1-bis(4-chlorophenyl)-2,2-dichloroethane; TDE; tetrachlorodiphenylethane
Chemical formula	$C_{14}H_9Cl_5$	$C_{14}H_8Cl_4$	$C_{14}H_{10}Cl_4$
Chemical structure			
Melting point	109 °C	89 °C	109-110 °C
Boiling point	Decomposes	336 °C	350 °C
Water solubility	0.025 mg/L at 25 °C	0.12 mg/L at 25°C	0.090 mg/L at 25 °C
Partition coefficients:			
Log K_{ow}	6.91	6.51	6.02
Log K_{oc}	5.18	4.70	5.18
Vapor pressure	1.60×10^{-7} mmHg at 20 °C	6.0×10^{-6} mmHg at 25 °C	1.35×10^{-6} mmHg at 25 °C

Table 3-D Endosulfan properties

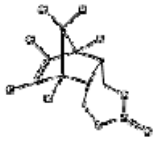
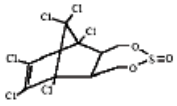
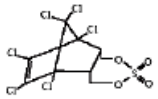
Characteristic	Endosulfan I (α-endosulfan)	Endosulfan II (β-endosulfan)	Endosulfan sulfate
Synonym(s)	6,9-methano-2,4,3- benzodiozathie-pin-6,7,8,9,10,10-hexachloro-1,5,5a,6,9, 9a-hexahydro-3-oxide	6,7,9,10,10-hexachloro-1,5,5a,6,9, 9a-hexahydro-6,9-methano-2,4,3-benzodiozathiepin-3-oxide	6,7,8,9,10,10-hexachloro-1,5,5a,6,9, 9a-hexahydro-6,9-methano-2,4,3-benzodiozodiozathiepin-3,3-dioxide
Chemical formula	$C_9H_8Cl_6O_3S$	$C_9H_8Cl_6O_3S$	$C_9H_6Cl_6O_4S$
Chemical structure			
Melting point	108-110 °C	207-212 °C	181 °C, 198-201 °C
Boiling point	No data	No data	No data
Water solubility	0.53 mg/L at 25 °C	0.28 mg/L at 25°C	0.22 mg/L at 25 °C
Partition coefficients:			
Log K_{ow}	3.83	3.52	3.66
Log K_{oc}	3.55	No data	No data
Vapor pressure	1×10^{-5} mmHg at 25 °C	1×10^{-5} mmHg at 25 °C	1×10^{-5} mmHg at 25 °C

Table 4-D Endrin and endrin aldehyde properties

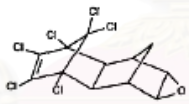
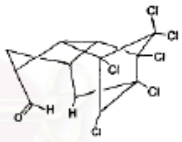
Characteristic	Endrin	Endrin aldehyde
Synonym(s)	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4A,5,6,7,8,8A-octahydro-endo,endo-1,4:5,8-dimethanonapthalene	1,2,4-methanecyclopenta(c,d)pentalene-5-carboxaldehyde,2,2a,3,3,4,7-hexachlorodecahydro
Chemical formula	$C_{12}H_8Cl_6O$	$C_{12}H_8Cl_6O$
Chemical structure		
Melting point	235 °C, 226-230 °C	176-177 °C
	49-60 °C (technical grade)	95 °C (technical grade)
Boiling point	Decomposes at 245 °C	No data
	Decomposes above 200 °C	
Water solubility	200 µg/L at 25 °C	50 µg/L, 0.25-0.26 ppm at 25°C
Partition coefficients:		
Log K_{ow}	5.6, 5.34, 5.45 (calculated)	3.146, 4.7, 5.6 (calculated)
Log K_{oc}	4.532 (calculated), 5.195 (±0.005)	4.80, 3.929-4.653 (calculated)
Vapor pressure	2.0×10^{-7} mmHg at 25 °C	2.0×10^{-7} mmHg at 25 °C

Table 5-D Heptachlor and heptachlor epoxide properties

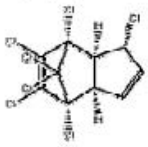
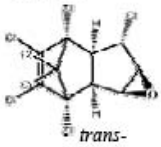
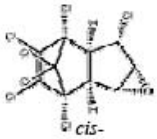
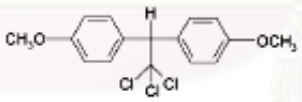
Characteristic	Heptachlor	Heptachlor epoxide
Synonym(s)	3-Chlorochlordene; 1,4,5,6,7,8,8a-heptachloro-3a, 4,7,7a-tetrahydro-4,7-methanoindane; 1,4,5,6,7,8,-8-heptachloro-3A,4,5,5a tetrahydro; alpha-dicyclo-pentadiene, 3,4,5,6,8,8a-heptachloro, and others	Epoxyheptachlor; 1,4,5,6,7,8,8a-hepta-chloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene; 4,7-methanoindan,1,4,5,6,7,7-heptachloro-1a,1b,5,5a,6,6a-hexahydro
Chemical formula	$C_{12}H_8Cl_6O$	$C_{12}H_8Cl_6O$
Chemical structure		 *trans-*  *cis-*
Melting point	95-96 °C (pure); 46-74 °C (technical grade)	160-161.5 °C
Boiling point	145 °C	No data
Water solubility	0.05 mg/L at 25 °C	0.275 mg/L at 25°C
Partition coefficients:		
Log K_{ow}	6.10	5.40
Log K_{oc}	4.34	3.34-4.37
Vapor pressure	3×10^{-4} mmHg at 20 °C and 25 °C	2.6×10^{-5} mmHg at 25 °C

Table 6-D Methoxychlor properties

Characteristic	Methoxychlor
Synonym(s)	2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane; 1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane; methoxy-DDT; 1,1-(2,2,2-trichloroethylidene)-bis(4-methoxybenzene)
Chemical formula	$C_{16}H_{15}Cl_3O_2$
Chemical structure	
Melting point	89 °C, 77 °C (technical grade)
Boiling point	No data (decomposes)
Water solubility	0.045 mg/L at 25 °C 0.02 mg/L at 15 °C 0.04 mg/L at 24 °C 0.095 mg/L at 35 °C 0.185 mg/L at 45 °C
Partition coefficients:	
Log K_{ow}	4.68-5.08
Log K_{oc}	4.9
Vapor pressure	1.4×10^{-6} mmHg at 25 °C

APPENDIX E

ORGANOCHLORINE PESTICIDES STATUS IN THAILAND

Table 1-E Organochlorine pesticides status in Thailand

Common Name	Chemical Name	Molecular Formula	Imported Year*	Banned Year**
Aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo,exo-5,8-dimethanonaphthalene	$C_{12}H_8Cl_6$	1975	1988
Benzene hexachloride (BHC) or Hexachlorocyclo hexane (HCH)	Hexachlorocyclohexane	$C_6H_6Cl_6$	1975	2001
Chlordane	1, 2, 4, 5, 6, 7, 8, 8-Octachloro – 2 , 3, 3a, 4, 7, 7a – hexahydro – 4, 7 - methanoindene	$C_{10}H_6Cl_8$	1974	2000
DDD	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane	DDT derivative	No record	2001
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene	$C_{14}H_8Cl_4$	No record	-
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane	$C_{14}H_9Cl_5$	1978	1983
Dicofol	1,1-bis(4′-chlorophenyl)2,2,2-tricholoro-ethanol	$C_{14}H_9Cl_5O$	1975	-
Dieldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,6,8,8a-hexahydro-6,7-epoxy-1, 4:5, 8-dimethanonaphthalene	$C_{12}H_8Cl_6O$	1975	1988
Endosulfan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide	$C_9H_6Cl_6O_3S$	1975	2004
Endrin	(1a,2,2a,3,6,6a,7,7a)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth[2,3-b] oxirene	$C_{12}H_8Cl_6O$	1978	1981
Heptachlor	1, 4, 5, 6, 7, 8, 8 - Heptachloro - 3a, 4, 7, 7a – tetrahydro - 4, 7 - methanoindene	$C_{10}H_5Cl_7$	1978	1988
Methoxychlor	1,1,1-trichloro-2,2-di-(*p*-methoxyphenyl) ethane	$C_{16}H_{15}O_2Cl_3$	No record	-

Source: Thirakupt *et al.*, 2007.

APPENDIX F

QUESTIONNAIRE-BASED DIETARY SURVEY

FOR RISK ASSESSMENT

The Questionnaire-based dietary survey for risk assessment form:

Interviewer Name: ..... ***Questionnaire No.:*** .....

Date...../...../.....

National Research Center for Environmental and Hazadous Waste Management Chulalongkorn University

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Questionnaire for Human Health Risk Assessment of Local Community at Khlong 7, Rangsit Agricultural Area, Pathum Thani Province.

Please answer the question and/or mark (✓) in the blank

1. Name..... Surname..... .....

2.Address.....

.....

3. Sex

( ) Male ( ) Female

4. Age..... ....kg.

5. Weight.....kg.

6. Years Lived in Khlong 7

( ) 0-5 years ( ) 6-10 years ( ) 11-15 years ( ) 16-20 years

( ) 21-25 years ( ) 26-30 years ( ) > 30 years

7. Have you ever consumed aquatic organisms in Khlong 7 before?

( ) Yes (go to question 8) ( ) No (end of the interview)

8. What kind of aquatic organisms do you consume? (*Conti.*)

8.1 Morning Glory (*Ipomomea aquatica* Forssk.)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8.2 Neptunia (*Neptunia oleracea* Lour.)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8.3 Water Lily (*Nymphaea lotus* L.)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8.4 Lanchester's freshwater Prawn (*Macrobrachium lanchesteri*)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8.5 Freshwater Snail (*Filopaludina mertensi*)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8.6 Snakehead (*Channa striatus*)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8. Which kind of aquatic organisms do you consume? (*Conti.*)

8.7 Nile Tilapia (*Orechromis niloticus*)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8.8 Marbled Sleeper (*Oxyeleotris marmoratus*)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8.9 Bronze Featherback (*Nototerus notopterus*)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8.10 Silver Barb & Red-tail Tinfoil Barb

(*Puntius gonionotus & Puntius altus*)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8.11 Moonbeam Gourami & Three-spot Gourami

(*Trichogaster microlepis & Trichogaster trichopterus*)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

8. Which kind of aquatic organisms do you consume? (*Conti.*)

8.12 Climbing Perch & Catopra

(*Anabas testudineus & Pristolepis fasciata*)

( ) Yes ( ) No

Amount of consumption .....kg/day

Consumption per month.....

End of Interview

⁂⁂⁂⁂⁂ ***Thank You*** ⁂⁂⁂⁂⁂

Notes:

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BIOGRAPHY

Wattasit Siriwong was born on the 22 January, 1975, in Songkhla province. He received a Bachelor of Science degree in Biotechnology in 1997 from the Faculty of Science, Mahidol University and Master of Environmental Science in 2001 from the Faculty of Science and Technology, Thammasat University. He continued his study in the International Postgraduate Program in Environmental Management for a Doctor of Philosophy in Environmental Management at the Graduate School, Chulalongkorn University in 2004 and completed the program in 2007.