OPTIMIZATION OF SOLID-PHASE MICROEXTRACTION FOR THE DETERMINATION OF ORGANOPHOSPHORUS AND ORGANOCHLORINE PESTICIDE RESIDUES IN NATURAL WATER NEAR AQUACULTURE FARMS

CHAN CHUN FOONG

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2010

OPTIMIZATION OF SOLID-PHASE MICROEXTRACTION FOR THE DETERMINATION OF ORGANOPHOSPHORUS AND ORGANOCHLORINE PESTICIDE RESIDUES IN NATURAL WATER NEAR AQUACULTURE FARMS

BY

CHAN CHUN FOONG

A DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2010

ABSTRAK

Optimasi Solid-Phase Microextraction Teknik untuk Pentafsiran Kehadiran Racun Serangga Organofosforus dan Organoklorin dalam Air Alam yang berkaitan dengan Aktiviti-aktiviti Akuakultur

oleh,

Chan Chun Foong

Penyelia utama: Profesor Madya Dr. Richard Wong Chee Seng

Penyelia kedua: Profesor Dr. Tan Guan Huat

Industri penternakan udang mengalami perkembangan yang mendarat pada tahun 80-an, akibat daripada permintaan yang tinggi dari seluruh dunia. Selain daripada kebinasaan kawasan bakau, kegiatan penternakan udang yang intensif telah mengakibatkan kualiti air di persekitaran kawasan penternakan yang terlibat semakin merosot. Pelbagai jenis bahan-bahan kimia dan produk-produk biologi telah digunakan untuk mengawal keadaan kolam-kolam penternakan udang daripada kejangkitan penyakit. Racun serangga digunakan sebagai pembasmi kuman untuk membunuh organisma-organisma yang tidak dikehendaki. Kaedah SPME-GC-ECD telah dikaji untuk mentafsirkan kehadiran-kehadiran azinphos-ethyl, chlorphyrifos-methyl, diazinon, dichlorvos, endosulfan-I, endosulfan-II, endosulfan sulfate dan malathion di dalam sampel-sampel air yang diperolehi dari persekitaran kawasan penternakan udang di daerah Manjung, Perak. Kaedah SPME yang optima telah ditentukan dan alat GC-ECD telah digunakan untuk penganalisaan analit yang terpilih. Pengekstrakan selama 30 minit pada suhu 40°C di bawah pengacauan berterusan dan perlepasan bahan ekstrak secara terma pada suhu 270°C selama 12 minit telah digunakan. Tiada pengubahsuaian matrix sampel digunakan dalam kajian ini. Pemulihan

ekstrak diperolehi untuk semua analit adalah dalam lingkungan 90.64% hingga 124.29% manakala LOD yang diperolehi adalah dalam lingkungan 0.01ppb hingga 5ppb. Chlorphyrifos-methyl, diazinon, endosulfan-I, endosulfan-II, endosulfan sulfate dan malathion telah dikesan manakala azinphos-ethyl dan dichlorvos tidak dapat dikesan.

ABSTRACT

Optimization of Solid-Phase Microextraction for the Determination of Organophosphorus and Organochlorine Pesticide Residues in Natural Water near Aquaculture Farms

by,

Chan Chun Foong

Supervisor: Associate Professor Dr. Richard Wong Chee Seng

Co-supervisor: Professor Dr. Tan Guan Huat

The global shrimp farming industry had a rapid growth in the 1980s due to the high demand for shrimp. Apart from the destruction of mangroves and wetlands, the intensive operation of shrimp aquaculture deteriorated the water quality in the region. Variety of chemicals and biological products were used to prevent the outbreaks of diseases in the intensively managed shrimp ponds. Pesticides were used as disinfectands to kill unwanted organisms in the pond. SPME-GC-ECD method was developed for the determination of azinphos-ethyl, chlorphyrifos-methyl, diazinon, dichlorvos, endosulfan-I, endosulfan-II, endosulfan sulfate and malathion from water samples of shrimp aquaculture area in Manjung district, Perak. The optimum SPME method developed for GC-ECD analysis of selected analytes was found to be 30 min of extraction at 40°C under continuous stirring condition; 12 min of desorption at 270°C. No matrix modifications were applied in this study. Recoveries obtained ranged between 90.64% and 124.29% while the limit of detection (LOD) ranged from 0.01 to 5ppb for the targeted compounds. Chlorphyrifos-methyl, diazinon, endosulfan-I, endosulfan-II, endosulfan sulfate and malathion were detected from the water samples whilst azinphos-ethyl and dichlorvos were not detected.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my supervisors, Assoc. Prof. Dr. Richard Wong Chee Seng and Prof. Dr. Tan Guan Huat for their invaluable advice, guidance, patience and time spent throughout the entire research.

I will never forget the precious moment in the laboratory that shared together with my colleagues, Ms Ooi Mei Lee, Ms Heong Chee Yin and Ms Elizabeth Fong. Thanks for their assistance and those valuable sharing.

I also sincerely appreciate family of Assoc. Prof. Dr. Richard Wong Chee Seng as well as family of Ms Ooi Mei Lee for their warmest hospitality throughout the sampling trips to Manjung district, Perak.

Special thanks to my husband, Mr Chuah Hoon Pong, my parents and friends for their constant support and encouragement throughout my study period.

Last but not least, I wish to thank University of Malaya for providing the financial assistance, Pasca Siswazah and the facilities to accomplish this study.

Thank you!

TABLE OF CONTENTS

Contents **Page**

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

$^{0}/_{00}$	part per thousand (unit for salinity)
°C	degree Celsius
AFID	alkali flame ionization detector
ANSI	American National Standards Institute
BSI	British Standard Institution
DO	dissolve oxygen
ECD	electron capture detector
EDTA	ethylenediaminetetraacetic acid
ESA	Ecological Society of America
FAO	Food and Agricultural Organization
FID	flame ionization detector
FPD	flame photometric detector
GC	gas chromatography
GLC	gas-liquid chromatography
GPS	global positioning system
ISO	International Organization for Standardization
LLE	liquid-liquid extraction
LOD	limit of detection
Log P_{ow}	octanol-water coefficients
LOQ	limit of quantitation
mg/l	miligram per litre
min	minute
MS	mass spectrometry
NaCl	sodium chloride
NPD	nitrogen-phosphorus detector
PARs	peak area ratios
PDMS	poly(dimethylsiloxane)
ppb	part per billion
R^2	correlation coefficient
rpm	round per minute
RSD	relative standard deviation

SD	standard deviation
SPE	solid-phase extraction
SPME	solid-phase microextraction
TCD	thermal conductivity detector
TID	thermionic detector
USSR	Union of Soviet Socialist Republic
WCOT	wall-coated open tubular

CHAPTER 1 – INTRODUCTION

1.1 Aquaculture

With an increasing demand for food, energy and space by growing population, the pressure of exploitation is reaching alarming levels on an increasing number of species and over an expanding area. Ocean is believed to be the most potential region to be explored for alternative food source as two-third of the earth is covered by water. However, fishery resources nowadays in general are heavily exploited. With the increasing recognition of the extent of decline of the world's fisheries, it is apparent that aquaculture is a potential means of relieving pressure on fish stocks and also an important source to meet the demand for fish products. Thus, expectations for aquaculture to increase its contribution to the world's production of aquatic food are very high.

At present, aquaculture is regarded worldwide as one of the fastest growing food-producing sub-sectors, demonstrated by a continuous increase in total production throughout the last decade or more, particularly in a number of developing countries (Ahmed and Lorica, 2002). According to Food and Agricultural Organization (FAO) statistics, aquaculture's contribution to global supplies of fish, crustaceans and molluscs continues to grow, increasing from 3.9 percent of total production by weight in 1970 to 27.3 percent in 2000 (FAO, 2002). Aquaculture provided not only the protein supply but also the opportunity of employment as well as the foreign exchange income. It plays an important role as a complementary alternative to the outputs from the capture fishery sector and as a supplementary economic activity.

According to the statistic on aquaculture compiled by FAO 2004a, the contribution of aquaculture to global supplies of fish, crustaceans and molluscs continues to grow (Table 1.1).

Table 1.1: Top ten producers in aquaculture production: quantity and growth

Producer	**2000**	**2002**	**APR***
	(thousand tonnes)		**(percent)**
Top ten producer in terms of quantity			
China	24 580.7	27 767.3	6.3
India	1 942.2	2 191.7	6.2
Indonesia	788.5	914.1	7.7
Japan	762.8	828.4	4.2
Bangladesh	657.1	786.6	9.4
Thailand	738.2	644.9	-6.5
Norway	491.2	553.9	6.2
Chile	391.6	545.7	18.0
Vietnam	510.6	518.5	0.8
United States	456.0	497.3	4.4
Top ten subtotal	31 318.8	35 248.4	6.1
Rest of the world	4 177.5	4 550.2	4.4
Total	35 496.3	39 798.6	**5.9**

*APR = annual percentage rate in 2002

Source: FAO 2004, The State of World Fisheries and Aquaculture.

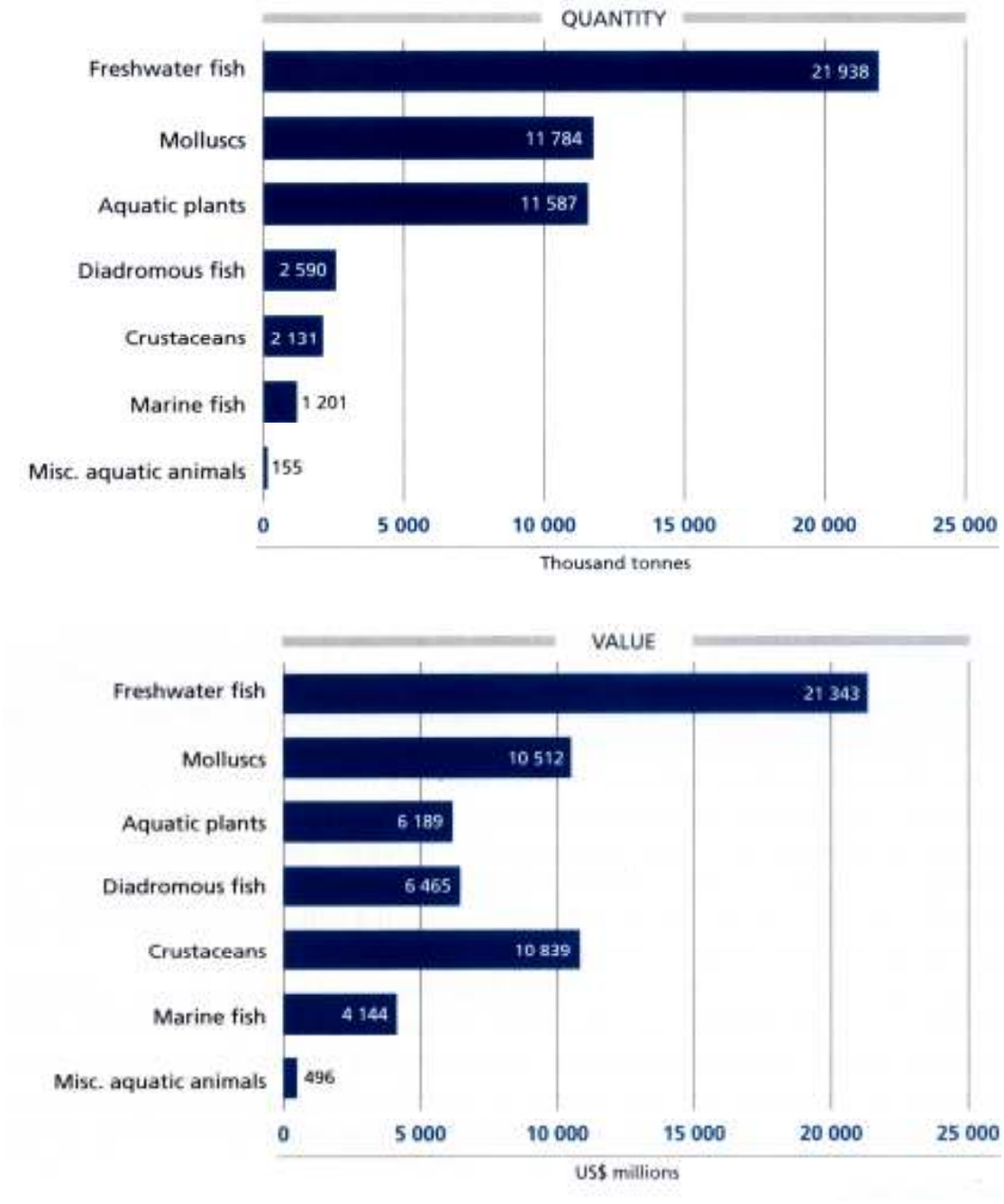


Figure 1.1: World aquaculture production: major species groups in 2002 (*Source:* FAO, 2004a)

In 2002, total world aquaculture production (including aquatic plants) was reported to be 51.4 million tonnes by quantity and US$60.0 million by value. Countries in Asia accounted for 91.2 percent of the production quantity and 82 percent of the value. Of the world total, China is reported to produce 71.2 percent of the total quantity and 54.7 percent of the total value of aquaculture production. The majority of aquatic organisms currently being cultured are representative of these species groups: finfish, molluscs, aquatic plants, and crustaceans (Figure 1.1) (FAO, 2004a).

The word "aquaculture" is defined by FAO as "The farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated. For statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture" (FAO, 1995).

On the other hand, Stickney (1994) defined "aquaculture" as "the rearing of aquatic organisms under controlled or semi-controlled conditions". More simply, aquaculture is underwater agriculture. In the broad sense, aquaculture includes the rearing of tropical fishes; the production of minnows, koi, and goldfish; the culture of sport fishes for stocking into farm ponds, streams, reservoirs, and even the ocean; the production of animals for augmenting commercial marine fisheries; and the growth of aquatic plants. Plants, such as single-celled algae, are considered to the extent that they may be necessary as a component of some or all of the life history stages of certain aquatic animals.

Consideration is also given to the control of nuisance aquatic vegetation, which includes rooted and floating plants as well as filamentous algae (Stickney, 1994).

It is widely believed that the culture of aquatic organisms had its beginning in Asia. Through the years, the region has remained in the forefront of aquaculture development and continues to produce the lion's share of the global aquaculture output. The Chinese are ancient masters of fish farming with a history dating back to the 5th century. Fish farming was common in East and Southeast Asia in the 13th and 14th centuries using inherited traditional farming techniques evolved through generations, many of which are still being practiced in many parts of Asia today (Joseph, 1990). Landau (1992) mentioned that the origins of aquaculture are not clear, but it was probably first practiced by either the Egyptians, who may have reared tilapia, or the Chinese, who grew carp. It then spread through Asia and Europe.

Aquaculture accounts for over 13 million tonnes of aquatic products harvested each year, and the industry is growing rapidly. It is extremely important in Asia, where carp, tilapia, yellow-tail, salmon, shrimp, and seaweeds are grown. In Central America, aquaculture is dominated by a very productive shrimp industry. In Europe, the Atlantic salmon, eels, trout, carp, oysters, and mussels are cultured in large numbers. In Canada, salmonids are the most culture species. In the United States, catfish, salmonids, baitfish, crawfish, and several species of molluscs also generate significant amounts of income (Landau, 1992).

1.2 Types of aquaculture

One means of distinguishing between aquaculture and the mere hunting and gathering of fish and shellfish is associated with the degree of control that is exerted by humans over the environment in which the organisms live. Instead of managing a water system and the various species it contains, to obtain an "optimum" or "sustainable" harvest, aquaculturists typically manage for maximum production of one or a small number of species. Attempts are made to eliminate, insofar as is possible, stress on the species being cultured and competition among the organisms of interest.

As increasing degrees of control over the environment are implemented by the aquaculturists, the level of intensity associated with the culture system is said to increase. Various types of aquatic production systems can be thought of as lying along a continuum of levels of production. Natural systems (e.g. a stream or lake) exist at one end, and recirculating water systems at the other (Figure 1.2).

For some of the types of water systems in between, it can be argued which is more intense than the other since production may be similar, but the level of technology required to develop and operate the systems can vary considerably. Even within a given type of culture system (e.g. ponds), there can be a considerable amount of variation in the level of intensity practiced by the culturist. Production within ponds is quite variable, depending on the management strategy that is employed (Stickney, 1994).

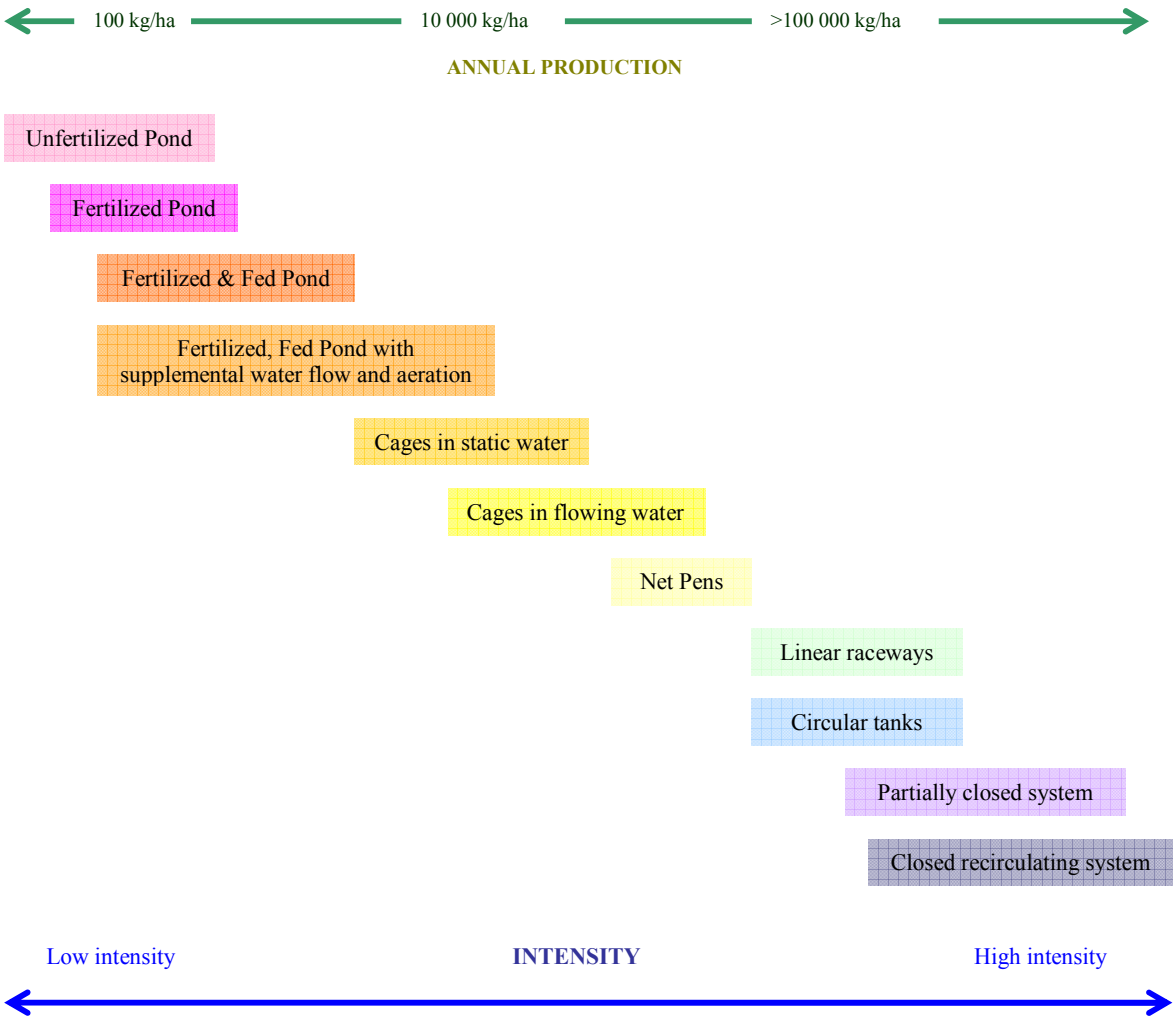


Figure 1.2: The intensity and production level continuum along which various types of aquaculture systems lie (Stickney, 1994).

Figure 1.2 shows that depending on the species under culture and various technological modifications in the various systems, their actual locations relative to one another and the range of production maxima for each can only be approximated (Stickney, 1994).

In general, aquaculture can be broadly classified as extensive, semi-intensive and intensive systems. Traditional extensive culture systems are characterized by low stocking densities and little or no supplementary feeding or fertilizer. The system relies on natural food

within the pond and tidal fluctuations for water exchange (Phillips *et al.*, 1993). The overall extensive systems are characterized by low inputs and low yields. Brackish water lagoons that have high levels of primary productivity or the cultivation of molluscs by spreading collected juveniles on the seabed would be good examples of extensive cultivation systems (Jennings *et al.*, 2001). Reservoirs and lakes are increasingly being used for stocking of tilapia and carps in China, India, Thailand and Sri Lanka. While Japan and China have undertaken marine farming of seaweeds, Japan and Taiwan have long been experimenting on ranching of penaeid shrimps in the sea (Joseph, 1990).

The progression from extensive to semi-intensive and intensive culture is marked by increasing inputs, of fertilizers or supplementary feed, supplementary stocking and improved water management. The semi-intensive aquaculture system includes the farming of finfishes, crustaceans, molluscs and seaweeds in ponds, cages, pens and other facilities. The culture systems can be further classified into two general types. The land-based culture systems involve farming of the above organisms in ponds and integrated aquaculture-agriculture systems. The main characteristic of the integrated farming system is its high dependence on primary productivity for fish production. The water-based culture systems involve the culture of finfish and/or crustaceans in cages and pens; molluscs and seaweeds suspended from floating rafts or stakes or on sea bottom or intertidal mudflats. The contribution of natural food is of significant importance especially for mollusc and seaweed farming (Joseph, 1990).

Intensive systems are defined by the most extreme levels of human control. In intensive systems there will be high stocking density, extensive use of artificial feeds that can be supplemented with vitamins, essential elements, antibiotics, and close environmental

control (Jennings, 2001). This cultural system involves the raising of carnivorous finfish and crustaceans in ponds, tanks, cages, raceways, silos, recirculating systems, etc., in which production entirely depends on the supply of formulated feed or trash fish. These systems are generally adopted for the production of high-priced commodities, in particular, shrimps, eels, seabass, catfish, grouper and salmon (Joseph, 1990).

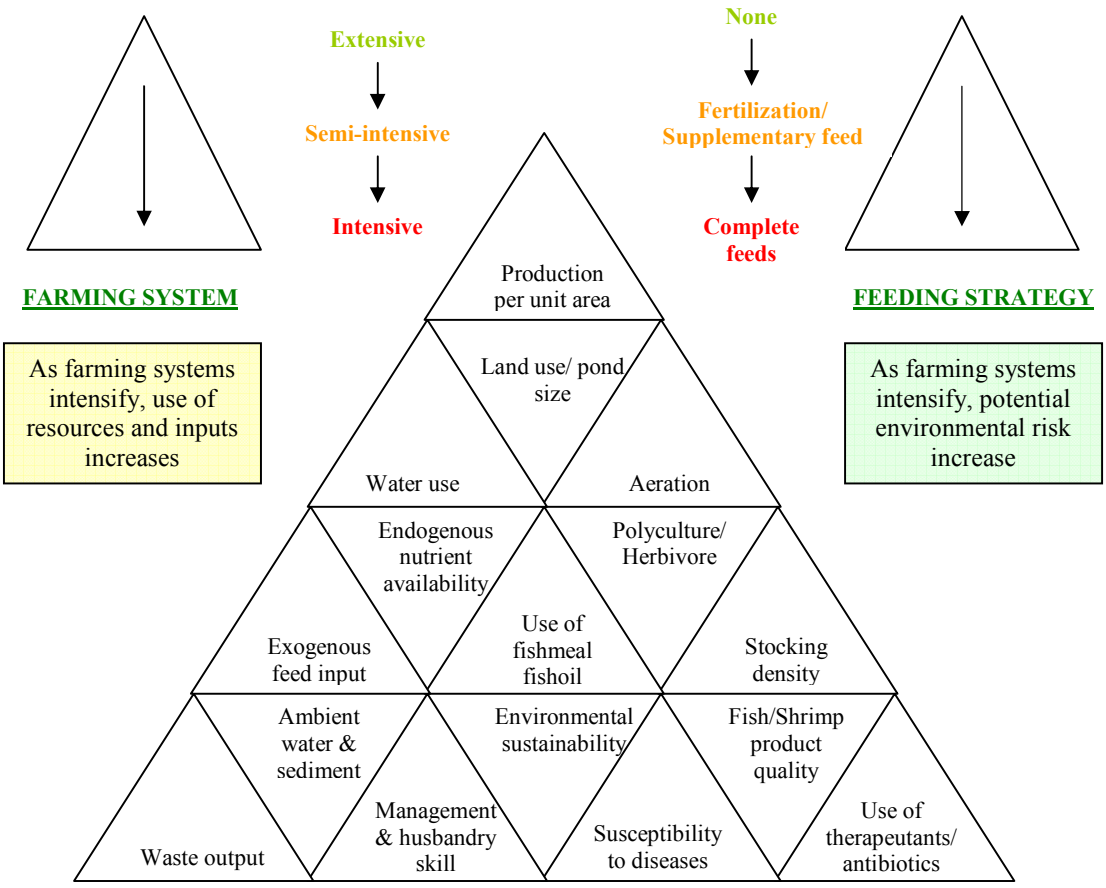


Figure 1.3: Main differences between conventional extensive, semi-intensive, and intensive farming systems in terms of resource use and potential environmental risk (Tacon *et al.*, 2003).

In general, the higher the intensity and scale of production, the greater the nutrient inputs required and consequent risk of potential negative environmental impacts emerging from

the aquaculture facility through water use and effluent discharge (Figure 1.3) (Tacon *et al.*, 2003).

1.3 Shrimp aquaculture

The global shrimp farming industry had a rapid growth in the 1980s mainly due to technological breakthroughs (such as hatchery and feed), high demand for shrimp resulting in high price and high profit of shrimp farming, and public support (Shang *et al.*, 1998).

In international trade, marine shrimp is the most prominent product from aquaculture industry, and aquaculture has been the major force behind increased shrimp trading during the past decade. Shrimp is already the most traded seafood product internationally, and about 26 percent of total production now comes from aquaculture. Since the late 1980s, farmed shrimp has tended to act as a stabilizing factor for the shrimp industry. The major markets are Japan, the United States and the European Community, and the largest exporters of farmed shrimp are Thailand, Ecuador, Indonesia, India, Mexico, Bangladesh and Viet Nam. Demand for shrimp and prawns are expected to increase in the medium to long term. Asian markets such as China, the Republic of Korea, Thailand and Malaysia will expand as local economies grow and consumers demand more seafood (FAO, 2002).

In 1998, the world's shrimp farmers produced an estimated 840 200 metric tons of whole shrimp in an operating area of 999 350 ha (Figure 1.4) (Páez-Osuna, 2001a). The Asian region produced the largest amount of cultured shrimp followed by Latin America. From 1975 to 1985, the production of farmed shrimp increased 300 percent; from 1985 to 1995, 250 percent.

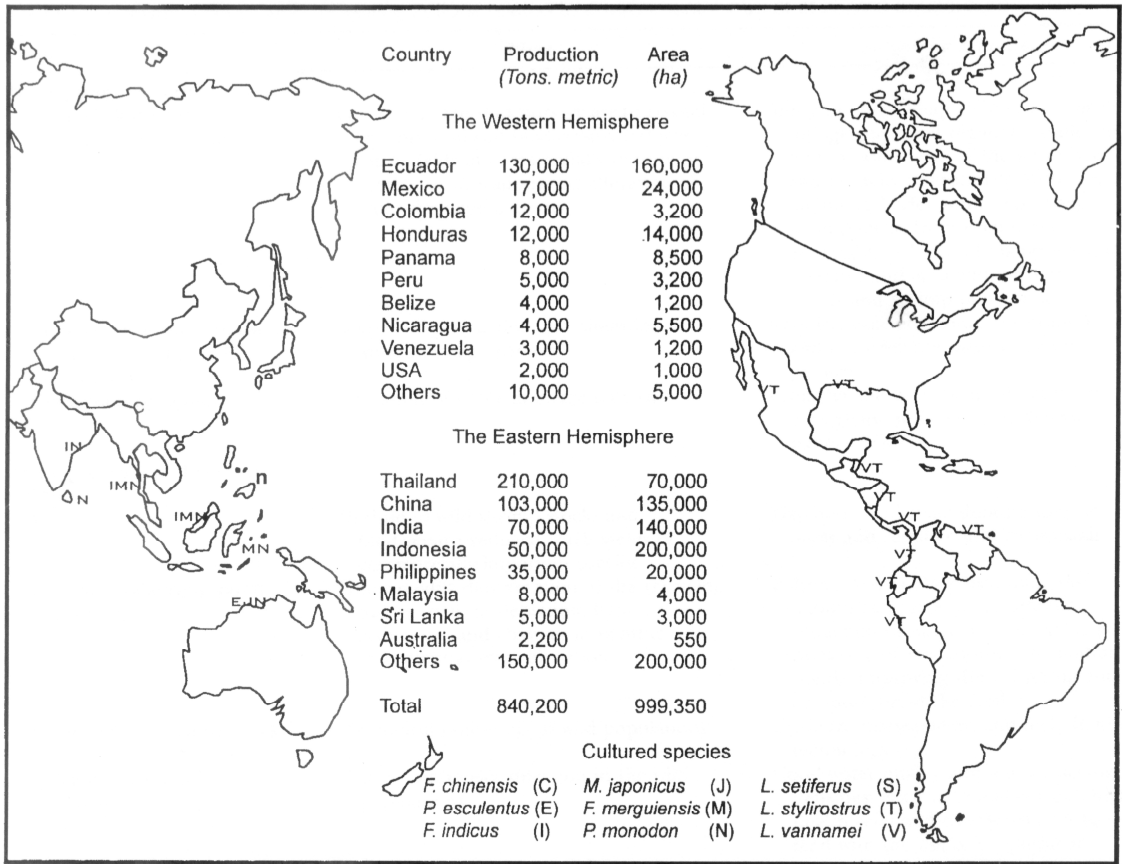


Figure 1.4: Shrimp species cultured and worldwide 1998 production by country (Páez-Osuna, 2001).

Worldwide, *Penaeus monodon* or known as black tiger shrimp and *Penaeus vannamei* or western white shrimp are the important species farmed (Stickney, 1994; Shang *et al.,* 1998; Gräslund and Bengtsson, 2001; INFOFISH International, 2001). The species dominating the marine shrimp culture in Southeast Asia are penaeid shrimp, especially *Penaeus monodon* (Gräslund and Bengtsson, 2001). The culture of brackish water and marine penaeid shrimps has a long history in Asia and some parts of Latin America (Aksornkoae, 1993).

Traditional culture systems are characterized by extensive aquaculture which the activities are primarily a coastal activity utilizing the mangroves as nursery grounds and nutrient or food supplies. However, the production gained from extensive aquaculture is low. This practice is inefficient and wasteful. As a result, intensified and semi-intensified aquacultures are emphasized to maximize yields and benefits where better management of aquaculture farming has been introduced (Aksornkoae, 1993). In some countries, such as Taiwan, limited land availability and the high cost of land has stimulated development of more intensive shrimp culture (Phillips *et al*, 1993; Shang *et al.*, 1998). Shrimp farms in Malaysia are classified as extensive and semi-intensive types of operation (Shang *et al.*, 1998).

The sitting of shrimp ponds is governed by many factors, including climate, elevation, water quality, soil type, vegetation, supply of postlarvae, support facilities and legislative aspects. As a result, ponds for shrimp culture have been constructed in a variety of different habitats, including salt pans, rice paddies, sugar fields, other agricultural land, abandoned coastal land and mangrove forests. A substantial area of shrimp ponds in Asia has been constructed on mangrove forests (Phillips *et at.*, 1993; Páez-Osuna, 2001a; Primavera, 2005) due to the function of mangroves as important nursery areas for many commercially important shrimp species throughout the tropics (Rönnbäck, 1999).

1.4 Environmental impact from shrimp aquaculture

After an impressive growth phase, shrimp aquaculture has created various socio-economic and environmental problems in many countries as numerous shrimp farmers often seek to maximize their short-term gain at the expense of the environment.

The most evident impact of and major concern for shrimp aquaculture is the destruction of mangroves and wetlands in the construction of shrimp ponds (Phillips, 1993; Dewalt *et al.*, 1996; Páez-Osuna, 2001a). Mangroves forests constitute the basis of the estuarine trophic system. They provide protection for shorelines in preventing coastal erosion, serve as a breeding, nursery and forage ground for many species of fish, animals, and shellfish, and provide habitat for large numbers of migratory and endemic species (Dewalt *et al.*, 1996; Rönnbäck, 1999; Primavera, 1998; Alonso-Pérez *et al.*, 2003).

Primavera (1998) asserted that mangroves have declined worldwide, particularly in South-east Asia, where losses have reached 70% - 80% in the last three decades. In the Philippines and Indonesia, mangrove removal has been traced mainly to the development of fish and shrimp ponds, as well as to agriculture, industry, residential uses, and local exploitation.

The operation systems in most of the world shrimp aquaculture are semi-intensive and intensive systems due to the profitability of the operation mode. Thus, the main input in most semi-intensive and intensive fish culture systems is fish feed, which is partly transformed into fish biomass and partly released into the water as suspended organic solids or dissolved matter such as carbon, nitrogen and phosphorus, originating from surplus food, faeces and excretions vial gills and kidneys. Other pollutants are residuals of drugs used to cure or prevent diseases (Phillips, 1993; Tovar *et al.*, 2000; Tacon *et al.*, 2003). These pollutants have deteriorated the water quality in the region. Gräslund and Bengtsson (2001) stated that intensively farmed shrimp ponds are often abandoned after 2 – 10 years due to environmental and disease problems caused by the accumulation of nutrients, declined access to clean water, etc. or simply because of lowered yields or profits.

Dewalt (1996) and Páez-Osuna *et al*. (2003) stated that during 1980s, the Gulf of Honduras and the Gulf of California experienced a boom in shrimp aquaculture and became the second largest producer in the western hemisphere. However, the development of this industry has been accompanied by concern about the destruction of mangrove forest, depletion of fishing stocks, disappearance of seasonal lagoons and deteriorating water quality.

1.5 Objective

The sustainable development of aquaculture activities depend heavily on the water quality that the aquatic organisms exposed to. However, the aquaculture activities itself may also degrade the water quality due to the wastes released and chemicals employed to the surrounding water environment.

The objective of this study is to access the water quality along the Sg. Manjung and its tributaries located in the state of Perak, Malaysia where increasing shrimp aquaculture activities are taking place.

The research carried out in this study will be focusing on the assessment of the residue levels of targeted pesticides that used in aquaculture by means of solid phase microextraction (SPME) technique. An optimized SPME method was developed and the extracted pesticides were then analyzed using Gas Chromatography-Electron Capture Detector (GC-ECD) technique.

CHAPTER 2 – LITERATURE REVIEW

2.1 Chemicals and biological products used in shrimp aquaculture

In intensively managed shrimp ponds, there is a high risk of disease outbreaks caused by virus, bacteria, fungi and other pathogens. The outbreak of viral diseases e.g. white spot disease, yellowhead disease, monodon baculo virus disease and hepatopancreatic parvo virus disease have had an impact on South-east Asian shrimp farming during the 1990s. The viral infections have caused severe economic losses in the region (Gräslund and Bengtsson, 2001). Taiwan (1987 – 1988), China (1993 – 1994), Indonesia (1994 – 1995), India (1994 – 1996), Ecuador (1993 – 1996), Honduras (1994 – 1997) and Mexico (1994 – 1997) have faced significant collapses in their shrimp production due to diseases (Páez-Osuna, 2001b).

There is a general trend towards intensification of production methods, and the quest for production gains is often accompanied by a great reliance on chemotherapeutants, feed additives, hormones, and more potent pesticides and parasiticides (GESAMP, 1997). Subasinghe *et al.* (1996) stated that chemicals increase production efficiency and reduce the waste of other resources. They assist in increasing hatchery production and feeding efficiency, and improve survival of fry and fingerlings to marketable size. They are used to reduce transport stress and to control pathogens, among many other applications (Subasinghe *et al.*, 1996). The most common products used in pond aquaculture are fertilizers and liming material. Disinfectants, antibiotics, algaecides, pesticides, and probiotics are also used to improve the production (Boyd and Massaut, 1999).

A field survey of chemicals and biological products used in marine and brackish water shrimp farming in Thailand had been carried out by Gräslund *et. al.* (2003). Among the chemicals and biological products used, soil and water treatment compounds were used by all farmers in the study and thereafter the most commonly used groups of products, in order of frequency of farmers using them, were pesticides and disinfectants, microorganisms, other feed additives, vitamins, antibiotics, fertilizers, and immunostimulants.

Chemicals and biological products used in shrimp farming are tabulated in Table 2.1. The applications of these chemicals and biological products are briefly discussed in this sub-chapter.

Table 2.1: Major category of chemicals used in aquaculture

Chemicals and their application
Chemicals associated with structural materials: plastic additives – stabilizers, pigments, antioxidants, UV absorbers, flame retardants, fungicides, and disinfectants; antifoulants – tributyltin
Soil and water treatments: flocculants – alum, EDTA, gypsum (calcium sulphate), ferric chloride; alkalinity control – lime/limestone; water conditioners/ammonia control – zeolite, *Yucca* extracts, grapefruit seed extract (KILOL); osmoregulators – sodium chloride, gypsum; hydrogen sulphide precipitator – iron oxide
Fertilizers: inorganic salts – limestone, marl, nitrates, phosphates, silicates, ammonium compounds, potassium and magnesium salts, trace element mixes; organic fertilizers – urea, animal and plant manures
Disinfectants: general – formalin, hypochlorite, iodophores – PVPI, sulphonamides, ozonation; topical – quaternary ammonium compounds, benzalkonium chloride
Antibacterial agents: β-lactams – amoxicillin; nitrofurans – furazolidone, nifurpirinol; macrolides – erythromycin, phenicols – chloramphenicol, thiamphenicol, florphenicol; quinolones – nalidixic acid, oxolinic acid, flumequine; rifampicin, sulphonomides, tetracyclines – oxytetracycline, chlortetracycline, doxycycline
Therapeutants and other antibacterials: acriflavine, copper compounds, dimetridazole, formalin, glutaraldehyde, hydrogen peroxide, levamisole, malachite green, methylene blue, niclosamide, potassium permanganate, trifluralin
Pesticides: ammonia, azinphos ethyl, carbaryl, dichlorvos, ivermectin, nicotine, organophosphates, organotin compounds, rotenone, saponin, trichlorofon, teased cake, mahua oil cake, derris root powder, lime, potassium permanganate, urea, triphenyltin, copper sulphate; Herbicides/algicides – 2,4-D, Dalapon, Paraquat, Diuron, ammonia,

copper sulphate, simazine, potassium ricinoleate, chelated copper compounds, food colouring compounds

Feed additives: acidifiers – citrates; antioxidants – butylated hydoxyanisole, butylated hydroxytoluene, ethoxyquin, propyl gallate; binders – animal protein, mineral (bentonite, magnesite), plant, seaweed, synthetic (urea formaldehyde, polyvinyl-pyrrolidone); feed enxymes; emulsifiers/surfactants – natural, synthetic; growth promoters – natural, synthetic; minerals – major and trace; pigments – food dyes, carotenoids (natural, synthetic); synthetic vitamins, amino acids and feeding attractants; immunostimulants, probiotics, mould inhibitors – natural, synthetic

Anaesthetics: benzocaine, carbon dioxide, metomidate, quinaldine, phenoxyethanol, tricaine methanesulphonate

Hormones: growth hormone, methyl-testosterone, oestradiol, ovulation-inducing drugs, serotonin

Fuels and lubricants: petroleum products – kerosene, petrol, diesel, oil

Environmental contaminants/pollutants: heavy metals/other metals – mercury, lead, mercury, arsenic, cadmium, chromium, copper, iron, manganese, nickel, selenium, silver, zinc; chlorinated insecticides – DDT, dieldrin, lindane and their degradation products; PCBs and Dioxins

Source: (Tacon *et al.,* 2003)

2.1.1 Soil and water treatment compounds

For soil and water treatment, alum and gypsum (calcium sulfate) is used to coagulate suspended colloids so that they will settle from the pond water (Boyd, 1995; GESAMP, 1997). Alum can also be used to remove phosphorus from aquaculture ponds. However, there are naturally plenty of ions in saltwater that enhance the sedimentation of particles and limit phosphate availability (Boyd, 1995). EDTA (ethylenediaminetetraacetic acid) reduces the bioavailability of the heavy metal ions and is used in larval rearing water in some shrimp hatcheries in South-east Asia and Latin America (GESAMP, 1997). Liming materials are used as amendments to shrimp ponds all over South-east Asia to neutralize the acidity of soil and water, and to increase the total alkalinity and total hardness. The most common liming materials are agricultural limestone (calcium carbonate, $CaCO_3$, dolomite, $CaCO_3 \bullet MgCO_3$; or calcium magnesium carbonate with another composition, $[CaCO_3]_{2-x}([MgCO_3]_x)$, calcium oxide (CaO) and calcium hydroxide ($Ca[OH]_2$)) (Primavera, 1993; GESAMP, 1997; Boyd. and Massaut, 1999). Zeolites are

aluminosilicate clay minerals which have a strong capacity to absorb or desorb molecules in internal cavities, and to exchange cations (Boyd, 1995).

2.1.2 Fertilizers

Fertilizers have a wide-spread use in shrimp ponds to increase the growth of natural food (Boyd and Massaut, 1999; GESAMP, 1997). There are two groups of fertilizers, i.e. organic and inorganic. The organic fertilizers used in South-east Asian shrimp farming are mainly chicken manure, but cow, water buffalo (carabao) and pig manure are also used (GESAMP, 1997; Primaver, 1993). Organic fertilizers are animal wastes or agricultural by-products which, when applied to ponds, may serve as direct sources of food for invertebrate fish food organisms and fish, or they may decompose slowly to release inorganic nutrients that stimulate phytoplankton growth (Boyd and Massaut, 1999). The most common inorganic fertilizers are nitrogen and phosphorus compounds, but potassium, trace metals, and silicate may be contained in some fertilizers (Boyd and Massaut, 1999). Fertilizers may be applied as individual compounds, or they may be blended to provide a mixed fertilizer containing two or more compounds (Boyd and Massaut, 1999).

2.1.3 Disinfectants/Antibacterial agents/Therapeutants

Disinfection, in the meaning of elimination of pathogens, can be obtained by heating, UV-radiation and a large number of chemical compounds (Gräslund and Bengtsson, 2001). Disinfectants can also be used to control phytoplankton or to oxidize the bottom soil (Gräslund and Bengtsson, 2001). Great quantities of disinfectants are used in intensive shrimp farming, both in hatcheries and in grow-out ponds. They are used for site and equipment disinfection and sometimes to treat disease (GESAMP, 1997). Calcium

hypochlorite ($Ca[OCl]_2$) and sodium hypochlorite (NaOCl) are the most commonly used disinfectants in South-east Asian shrimp farming (Primavera *et al*., 1993; GESAMP, 1997).

The application of hypochlorite is widely used in South-east Asia for viral control, either to disinfect incoming sea water before it is used in hatcheries, or to disinfect water or sediment in grow-out ponds (GESAMP, 1997). Hypochlorite is highly toxic to aquatic organisms (Gräslund and Bengtsson, 2001). Copper compounds have been used to eliminate external protozoans and filamentous bacterial diseases in post-larval shrimps. They are also used to inhibit phytoplankton growth and to induce moulting shrimps (Boyd , 1995; GESAMP, 1997). Formalin (or formaldehyde solution) is used worldwide in aquaculture (Primaver, *et. al.,* 1993). It is used as an antifungal agent and in the control of ectoparasites, primarily in hatchery systems, but also as a piscicide (GESAMP, 1997; Boyd and Massaut, 1999). Iodophores and quaternary ammonium coumpounds are used for the disinfection of water in grow-out ponds (Primavera *et al.,* 1993; GESAMP, 1997). Iodophores also used to disinfect the equipment used in aquaculture (Gräslund and Bengtsson, 2001).

Malachite green has been widely used in South-east Asian shrimp farming (Primavera *et al.*, 1993; GESAMP, 1997). It is used as therapeutants and antibacterial in aquaculture (Tacon *et al.,* 2003). Malachite green is prohibited in some South-east Asian countries such as Thailand, the USA and the European Union, due to its role as a respiratory enzyme poison (GESAMP, 1997). Ozonation is a disinfection technique often used in aquaculture (Primavera *et al.*, 1993; GESAMP, 1997). Ozonation is sometimes used to disinfect hatchery water, but less frequently to disinfect water in grow-out ponds (GESAMP, 1997).

2.1.4 Pesticides

The word 'pesticide' can be used in a broad sense to include disinfectants, or more specifically, for chemicals which target a certain group of organisms. The more specific pesticides can be used in shrimp ponds to kill organisms such as fish, crustaceans, snails, fungi, and algae (Gräslund and Bengtsson, 2001). Organochlorine compounds, in particular endosulfan (Thiodan), have been used in South-east Asian shrimp farming (GESAMP, 1997). Thiodan is still used occasionally in Thai marine shrimp farming (Gräslund and Bengtsson, 2001). Endosulfan is highly acute toxic to aquatic fauna (Brown, 1978; McEwen and Stephenson, 1979; Richardson, 1992; Leight and Van Dolah, 1999). Organophosphates are acetylcholinesterase inhibitor used as insecticides (McEwen and Stephenson, 1979; Emden and Peakall, 1996; Gräslund and Bengtsson, 2001). Organophosphates that have been use in South-east Asian shrimp farming are azinphos-ethyl (Gusathion A), diazinon, and trichlorfon (Dipterex) (GESAMP, 1997). Other organophosphates used in marine aquaculture are chlorpyrifos (Dursban), Dichlorvos, Demerin and Malathion (Gräslund and Bengtsson, 2001).

Organotin compounds were widely used in South-east Asia to remove molluscs before the stocking of shrimp ponds, but are now banned in the Philippines and Indonesia (GESAMP, 1997). Rotenone is derived from certain legumes, in South-east Asia primarily from *Derris elliptica*, where it is used to remove fish before stocking the shrimp ponds (GESAMP, 1997). Teaseed cake or saponin is often used in South-east Asia as a piscicide (Primavera *et al.*, 1993; Boyd and Massaut, 1999; GESAMP, 1997).

2.1.5 Feed additives

GESAMP (1997) stated that pigments, vaccines and immunostimulants have been successfully applied as feed additives for crustaceans. Immunostimulants have an increasing used globally to stimulate the non-specific immune system in shrimps (GESAMP, 1997). Vitamin B12, vitamin C and vitamin E are also added to shrimp feed (Gräslund and Bengtsson, 2001).

In many countries there is a widespread prophylactic use of antibiotics in shrimp hatcheries (GESAMP, 1997). In the text, the word antibiotics refers to biologically and synthetically produced substances (Gräslund and Bengtsson, 2001). Macrolides, nitrofurans, chloramphenicol, quilolones, rifampicin, sulfonamides and tetracyclines are the groups of antibiotics reported in the usage of shrimp farming (Gräslund and Bengtsson, 2001; Serrano, 2005).

Live bacteria inocula and fermentation products rich in extracellular enzymes are used in aquaculture (Boyd and Massaut, 1999). The reasons for using probiotics include the prevention of an off-flavor, reduce the proportion of blue-green algae, less nitrate, nitrite, ammonia, and phosphate; more dissolved oxygen, and an enhanced rate of organic matter degradation (Boyd, 1995).

2.1.6 Fuels and lubricants

As for any other farm operations, aquaculture production requires the use of fuel and lubricants for vehicles and power units used on the farm. Unless the materials are stored, used, and disposed in a proper way, they present both an environmental and safety hazard. Spill of fuels and lubricants can contaminate surrounding water and soil, or through runoff

find their way into pond waters. Fish and other aquatic animals exposed to petroleum products may develop characteristic off-flavors variously described as 'oily', 'diesel fuel', 'petroleum', or 'kerosene', and be rejected from the market (Boyd and Tucker, 1998).

2.2 Pesticides studied

Many aquaculture chemicals are, by their very nature, biocidal, and achieve their intended purpose by killing or slowing the population growth of aquatic organisms. Perhaps the greatest potential for ecological effects arises from the use of aquaculture chemicals to remove pest species from the surrounding environment.

Regulations in the United States regarding the use of biocides (pesticides and herbicides) have been implemented to ensure the safe use of those compounds. Nations other than United States may have more strict, or in many cases, very relaxed or nonexistent controls on the use of these chemicals. In many cases, chemicals designed for use in land-based agriculture have been applied to aquaculture systems without sufficient testing of the potential negative impacts those chemicals might have (Stickney, 1994).

There are eight compounds from pesticide groups of organochlorines and organophosphates will be determined in this study: azinphos-ethyl, chlorpyrifos-methyl, diazinon, dichlrovos, malathion, endosulfan I, endosulfan II and endosulfan sulfate. These compounds are chosen due to their usage in the aquaculture and their potential impact to the environment (Gräslund and Bengtsson, 2001; Tacon *et al.,* 2003).

2.2.1 Azinphos-ethyl

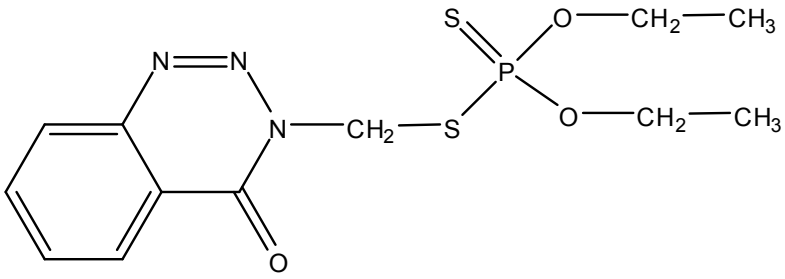


Figure 2.1: Azinphos-ethyl (Hayes and Laws, 1991)

Azinphos-ethyl is an organophosphorus pesticide. Chemical name of azinphos-ethyl is *S*-(3,4-dihydro-4-oxobenzo(*d*)-(1,2,3-triazin-3-ylmethyl-*O*,*O*-diethyl)phosphorodithioate. Azinphos-ethyl (BSI, ISO) is also known as the benzotriazine derivative of ethyl dithiophosphate, ethylguthion, and guthion (ethyl). In the USSR, the name is triazothion. Trade names include Athyl-Gusathion®, Azinfos-ethyl®, Azinos®, Azinophos-aethyl®, Crysthion®, Ethyl-azinophos®, Ethyl-Gusathion®, Gusation®, Gusathion®, and Bay 16255, Bayer 16259, ENT 22,014, and R1513. The CAS registry number is 2642-71-9 (Hayes and Laws, 1991).

Azinphos-ethyl has the empirical formula of $C_{12}N_{16}N_3O_3PS_2$ and a molecular weight of 354.4 g/mol. The pure material forms clear crystals having a melting point of 53˚C and a boiling point of 111˚C at 1 x 10^{-3} mm Hg. The density at 20˚C is 1.2384 g/cc. The vapor pressure of azinphos-ethyl is 2.2 x 10^{-7}mm Hg at 20˚C. Although azinphos-ethyl is not soluble in water, aliphatic hydrocarbons, or light petroleum, it is soluble in most other solvents. Azinphos-ethyl is thermally stable but is rapidly hydrolyzed by alkaline media (Hayes and Laws, 1991).

Azinphos-ethyl was introduced in 1953 by Bayer AG as a nonsystemic insecticide and acaricide. Although it is no longer registered for use in many countries due to its extreme acute toxicity to humans, some countries still use azinphos-ethyl for fruits and vegetables, pastures, cotton, cereals, coffee, potatoes, grapes, citrus, tobacco, rice, hops, and other crops of the forest industry. Azinphos-ethyl is available in 20% and 40% emulsifiable concentrates, 25% and 40% wettable powders, and 50% dusts (Hayes and Laws, 1991).

Biological effect LC_{50} (96 h) of azinphos-ethyl to molluscs is 0.12 mg/L and to fishes ranged between 19 µg/L and 80 µg/L while LC_{50} (48 h) of azinphos-ethyl to crustaceans ranged between 0.2 µg/L and 4 µg/L (Verschueren, 2001). The toxicity of azinphos-ethyl is Class Acute I which corresponds to highly acute toxic (Gräslund and Bengtsson, 2001).

2.2.2 Chlorpyrifos-methyl

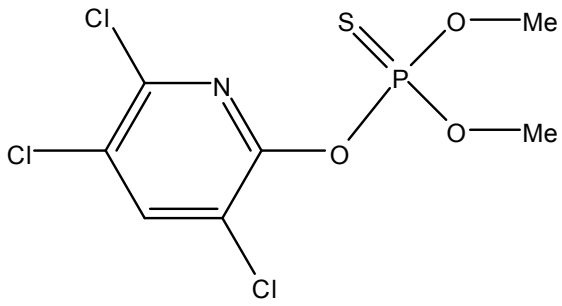


Figure 2.2: Chlorpyrifos-methyl (Hayes and Laws, 1991)

Chlorpyrifos-methyl is an organophosphorus pesticide. Chemical name for chlorpyrifos-methyl is *O*,*O*-dimethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothionate. Chlorpyrifos-methyl (ANSI, BSI, ESA, ISO) is also known by the trade names Dowco® 214 and

Reldan®. Code designations include ENT 27520. The CAS registry number is 5598-13-0 (Hayes and Laws, 1991).

Chlorpyrifos-methyl has the empirical formula of $C_7H_7Cl_3NO_3PS$ and a molecular weight of 322.51 g/mol. The pure material is a crystalline solid with a melting point of 45.5 – 46.5˚C. Its solubility in water is 5 ppm at 25˚C. The vapor pressure is 4.22 x 10^{-5} mm Hg at 25˚C. Chlorpyrifos-methyl decomposes 110 times more rapidly than chlorpyrifos at pH 5. It also has a greater tendency to undergo hydrolysis than chlorpyrifos. Chlorpyrifos-methyl is an insecticide and acaricide (Hayes and Laws, 1991).

Biological effects of chlorpyrifos-methyl at LC_{50} (36 h) to crustaceans is 0.00004 mg/L and LC_{50} (96 h) to fishes is 0.3 mg/L (Verschueren, 2001). The toxicity of chlorpyrifos-methyl is Class Acute I which corresponds to highly acute toxic (Gräslund and Bengtsson, 2001).

2.2.3 Diazinon

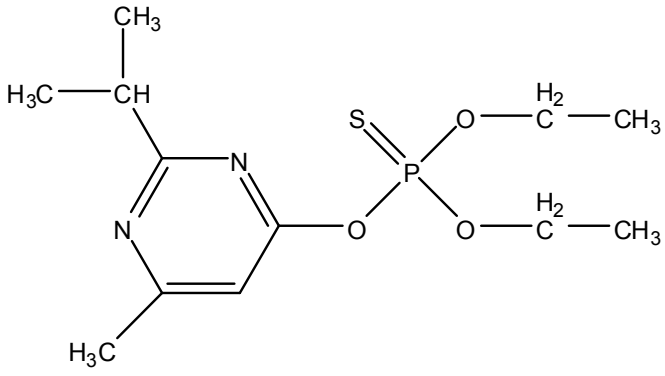


Figure 2.3: Diazinon (Hayes and Laws, 1991)

Diazinon is an organophosphorus pesticide. Chemical name of diazinon is *O*,*O*-diethyl *O*-(2-isopropyl-6-mehtyl-4-pyrimidinyl) phosphorothioate. The common name of diazinon (BSI, ESA, ISO) is in general use. Trade name include Basudin®, Diazitol®, Dipofene®, Neocidol®, Nucidol®, and Spectracide®. Code designations include G-24480 and OMS-469. The CAS registry number is 333-41-5 (Hayes and Laws, 1991).

Diazinon has the empirical formula of $C_{12}H_{21}N_2O_3PS$ and a molecular weight of 304.36 g/mol. The pure material forms a colorless liquid with a faint esterlike odor. The boiling point is 83 – 84˚C. The density at 20˚C is 1.116 – 1.118 g/cc. The vapor pressure is 1.4 x 10^{-4} mm Hg at 20˚C and 1.1 x 10^{-3} mm Hg at 40˚C. The refractive index (D^{20}) is 1.4798 – 1.4981. Diazinon is stable in alkaline formulations but is hydrolysed slowly by water and by dilute acids. Diazinon decomposes above 120˚C and is susceptible to oxidation. The solubility of diazinon in water at 20˚C is 40 ppm. It is miscible with alcohol, ether, petroleum ether, cyclohexane, benzene, and similar hydrocarbons (Hayes and Laws, 1991).

Biological effects of diazinon to crustaceans at LC_{50} (48 h) ranged from 0.9 to 2 µg/L while at LC_{50} (96 h) ranged between 2.57 and 200 µg/L (Verschueren, 2001). LC_{50} (96 h) of diazinon to fishes is ranged from 0.47 µg/L to 10.0 mg/L (Verschueren, 2001). Diazinon is considered moderate to highly acute toxic (Gräslund and Bengtsson, 2001).

2.2.4 Dichlorvos

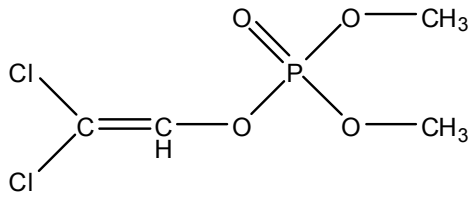


Figure 2.4: Dichlorvos (Hayes and Laws, 1991)

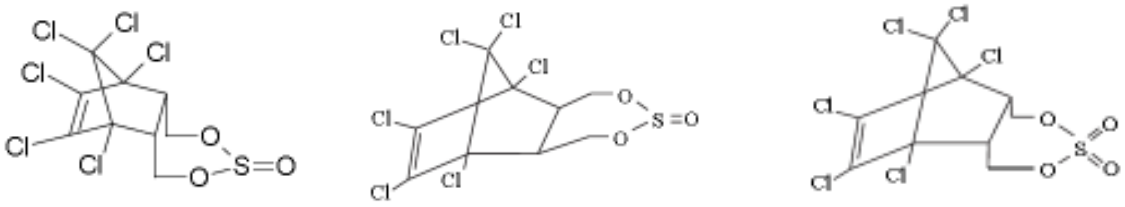
Dichlorvos is an organophosphorus pesticide. Chemical name of dichlorvos is *O*,*O*-dimethyl-*O*-2,2-dichlorovinyl phosphate. The common name of dichlorvos (BSI, ISO) generally is accepted, except in the USSR, where DDVF was used and dichlorfos is used now. The acronym DDVP was used extensively until supplanted by dichlorvos except in Japan. Trade name include Canogard®, Crossman's Fly-Cake®, Dedevap®, De-Pester Insect Strip®, Estrosol®, Hercol®, Herkol®, Kill-Fly Resin Strip®, Lethalaire®, Mafu®, Misect®, Nogos®, No-Pest Strip®, Nuvan®, Oko®, Phoracide®, Phosvit®, Vapona®, Vaponicide®, and Vaporette Bar®. The compound in the form of a resin granule formulation is sold as an anthelmintic under the names Atgard®, Dichloroman®, Equigard®, and Task®. Code designations include BAY-19149, ENT-20738, OMS-14, and SD-1750. The CAS registry number is 62-73-7 (Hayes and Laws, 1991).

Dichlorvos has the empirical formula $C_4H_7Cl_2O_4P$ and a molecular weight of 220.98. The pure material forms a colorless to amber liquid with a mild chemical odor. The density of dichlorvos at 25˚C is 1.415. The boiling point is 35˚C at 0.05 mm Hg. The vapor pressure of dichlorvos is 1.2 x 10^{-2} mm Hg at 20˚C. Dichlorvos is miscible with alcohol and in

aromatic and chlorinated hydrocarbon solvents. Its solubility is about 1% in water and 3% in kerosene and mineral oils. Dilute dichlorvos hydrolyses rapidly in the presence of moisture. A saturated aqueous solution (1%) hydrolyzes at a rate of about 3% per day. Concentrates are readily decomposed by strong acids and bases (Hayes and Laws, 1991).

Biological effects of dichlorvos to crustaceans at LC_{50} (96 h) ranged between 0.4 µg/L and 45 µg/L. While LC_{50} (48 h) of dichlorvos to crustaceans is 0.07- 0.26 µg/L. LC_{50} (96 h) of dichlorvos to fishes ranged between 200 µg/L and 3700 µg/L (Verschueren, 2001). The toxicity of dichlorvos to aquatic organisms is considered moderate to highly acute toxic (Gräslund and Bengtsson, 2001).

2.2.5 Endosulfans (Endosulfan I, Endosulfan II and Endosulfan sulfate)



Endosulfan I Endosulfan II Endosulfan sulfate

Figure 2.5: Endosulfan I, endosulfan II and endosulfan sulfate (Hayes and Laws, 1991)

Endosulfan is chlorinated hydrocarbon insecticide. Endosulfan is a mixture of two stereoisomers of 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide. Of the two isomers, α-endosulfan (endosulfan I) has the *exo* configuration and β-endosulfan (endosulfan II) has the *endo* configuration. Endosulfan sulfate is the derivative of endosulfan (Hayes and Laws, 1991).

The common name of endosulfan (ANSI, BSI, ISO) is in general use except in Iran and the USSR, where thiodan is used as a common name. Endosulfan was introduced in 1956 under code number Hoe-2671. Proprietary names include Beosit®, Cyclodan®, Malix®, Thifor®, Thimul®, and Thiodan®. Code designation for endosulfan have included FMC-5,462, Hoe-2671, OMS-204 (α-endosulfan), and OMS-205 (β-endosulfan). The CAS registry number is 115-29.7 (Hayes and Laws, 1991).

Endosulfan has the empirical formula of $C_9H_6Cl_6O_3S$ and a molecular weight of 406.95g/mol. The α isomer has a melting point of 109˚C and constitutes about 70% of the pure mixture. The β isomer has a melting point of 213˚C and constitutes about 30% of the mixture. Technical endosulfan contains 90 – 95% of the pure mixture; it is a brownish crystalline solid that smells of sulfur dioxide and melts at 70 - 100˚C. It is stable to sunlight. Endosulfan is hydrolyzed slowly by water and acids and rapidly by bases to the alcohol and SO_2. Its decomposition is catalyzed by iron, which it corrodes. Endosulfan is moderately soluble in most organic solvents but highly insoluble in water. The vapor pressure of technical endosulfan is 9×10^{-3} mm Hg, and its density is 1.745 g/cc (Hayes and Laws, 1991).

Endosulfan was first described in 1956 and was introduced as an experimental insecticide in the same year. It was first registered in the United States in 1960. It was formulated as emulsifiable concentrate, water-wettable powder, dust, and granules. Endosulfan has been used against a wide variety of agricultural pests but not against those of livestock, stored products, or the household (Hayes and Laws, 1991).

Biological effects of endosulfans to crustaceans at LC_{50} (96 h) ranged between 3.4 µg/L and 52.9 µg/L while at LC_{50} (48 h) ranged between 2.3 µg/L and 60 µg/L. LC_{50} (96 h) to fishes ranged between 0.09 µg/L and 5 µg/L (Verschueren, 2001). The toxicity of endosulfan considered highly acute toxic to aquatic organisms (Gräslund and Bengtsson, 2001).

2.2.6 Malathion

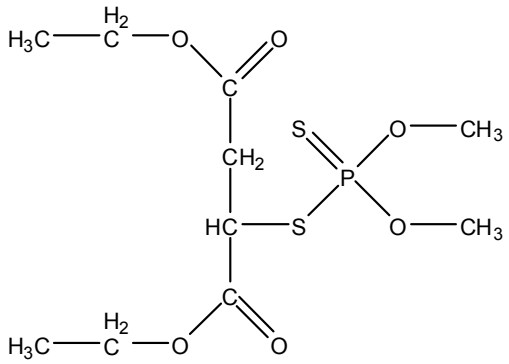


Figure 2.6: Malathion (Hayes and Laws, 1991)

About half of all organic phosphorus insecticides are dimethoxy compounds and malathion is one of them. Chemical name of malathion is *O*,*O*-dimethyl-*S*-(1,2-dicarbethoxyethyl)phosphorodithioate. The common name of malathion (BSI, CSA, ESA, ISO) is in general use. Other nonproprietary names include carbophos (USSR), maldison (Australia and New Zealand), and mercaptothion (Republic of South Africa). Trade names include Chemathion®, Cythion®, Emmaton®, Karbophos®, Malaspray®, Malathiozol®, Malathiozoo®, and Malathon®. Code designations include EI-4049, ENT-17034, and OMS-1. The CAS registry number is 121-75-5 (Hayes and Laws, 1991).

Malathion has the empirical formula of $C_{10}H_{19}O_6PS_2$ and a molecular weight of 330 g/mol. The pure material forms clear amber liquid with a boiling point of 156 - 157˚C at 0.7 mm Hg. The density is 1.23 g/cc at 25˚C. The vapor pressure is 4 x 10^{-5} mm Hg at 30˚C. The melting point of malathion is 2.85˚C. The solubility of malathion in water at room temperature is 145 ppm. It is miscible with many organic solvents, although its solubility in petroleum oils is limited. Malathion is rapidly hydrolyzed at pH above 7.0 or below 5.0 but is stable in aqueous solution buffered at pH 5.26. It is incompatible with alkaline pesticides (Hayes and Laws, 1991).

Malathion was introduced in 1950 by the American Cyanamid Company. The technical product is 95% pure. It is a nonsystemic insecticide and acaricide. Malathion is used in control of mosquitoes, flies, household insects, animal ectoparasites, and human head and body lice. Malathion is formulated as 25 – 86% emulsifiable concentrates, as 25 – 50% wettable powders, as dusts (usually at 4% concentration), and as ultralow-volume concentrates of 96% (Hayes and Laws, 1991). Biological effects of malathion to crustaceans at LC_{50} (96 h) is 1.0 µg/L (to *Gammarus lacustris* species). LC_{50} (96 h) to fishes ranged between 0.76 µg/L and 83 µg/L (Verschueren, 2001). The toxicity of malathion considered highly acute toxic to aquatic organisms (Gräslund and Bengtsson, 2001).

2.3 Potential impact of pesticides residues to the environment

Emphasis has been on the efficacy of the chemical on the target species, and there has been little consideration of the environmental effects of any chemical residues remaining in wastewater from the culture facility. In addition, there exist in general, the lack of knowledge concerning the effects and fates of chemicals and their residues in cultured organisms and within the aquaculture system itself.

For example, studies have been carried out in the Gulf of Fonseca, the South American region booming with shrimp aquaculture during 1980s. The potential threat to this region, and the shrimp and fisheries industries it supports may be contamination of the area by the misuse or indiscriminate use of pesticide. Independent studies carried out by a shrimp farm on the Purgatorio estuary found levels of lindane at 23 ppt and aldrin at 45.8 ppt. A small number of water, soil and clam tissue samples collected are also of concern. They indicated that all 10 water samples had detected levels of either heptachlor, aldrin, lindane, endosulfan or malathion. This indicates gross misuse of products and cause for concern since some of the levels approached the lethal concentration for aquatic environments. Two of the five tissue samples (clam) had accumulated detectable levels of endosulfan and aldrin (0.002 ppm). Two of the four soil samples taken from the estuarine zone of the Choluteca and Negro Rivers had detectable level of mevinphosphate, a pesticide used in the control of insects on fields, vegetables and fruit crops (Dewalt *et al*., 1996).

Concern is being expressed regarding the potential impact of aquaculture chemicals on the aquatic environment, adjacent terrestrial ecosystems and human health (FAO, 1997). Chemicals spread in the environment as a result of their use in aquaculture can be acutely

toxic, mutagenic or have other negative sub-lethal effects on the wild flora and fauna (Gräslund and Bengtsson, 2001).

Most available data on toxicity to aquatic life are from studies of freshwater organisms. A major concern regarding pollution from shrimp farms is the possible contamination of marine and brackish water ecosystems. However, freshwater can also be affected, both in coastal areas and in those inland areas where shrimp ponds are situated (Gräslund and Bengtsson, 2001). Hutchinson *et al.* (1998) compared the toxicity of chemicals to freshwater versus saltwater organisms. For the substances discussed in their study, chlorpyrifos was more toxic to saltwater than to freshwater fish, and endosulfan was clearly more toxic to saltwater than to freshwater invertebrates (Gräslund and Bengtsson, 2001). A reason for saltwater fish being more sensitive than freshwater fish to certain chemicals could be that they are not only exposed to contaminants in the water by way of the gills and skin, but also through osmoregulation by drinking the sea water (Hutchinson *et al.*, 1998).

The persistence of residues strongly depends on the environmental conditions. Major factors influencing the degradation are temperature, pH, the level of dissolved oxygen, light intensity and the presence of micro-organisms (GESAMP, 1997). Persistence is of major importance for the environmental effects of aquaculture chemicals. A significant persistence of a chemical, or its by-products, can influence organisms living in contact with the ponds and organisms in other ecosystems through bioaccumulation, biomagnifications or physical transport through air, water or soil (Gräslund and Bengtsson, 2001).

2.4 Analytical methods for the determination of pesticides residues in water

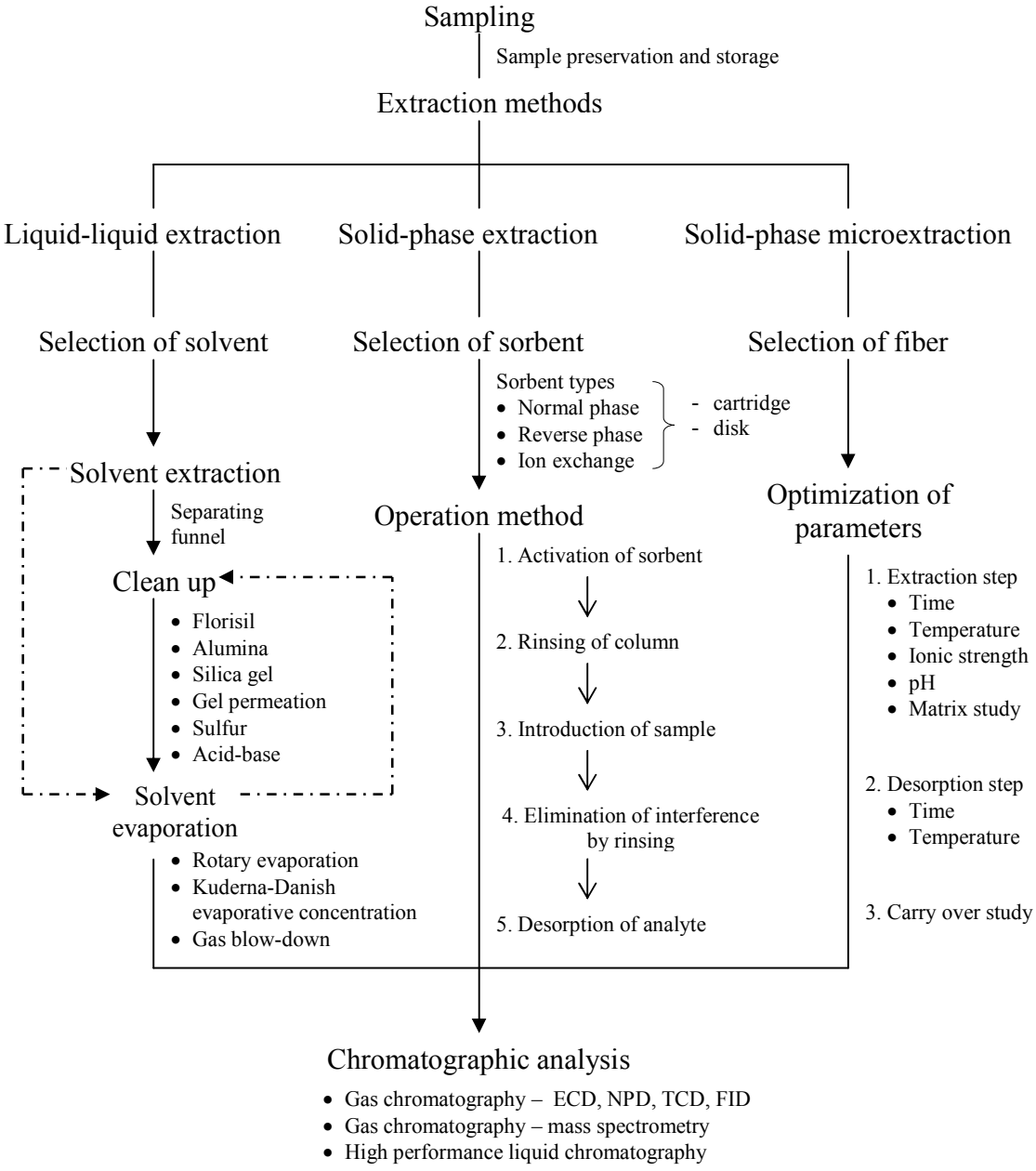


Figure 2.7: A diagram of the analytical steps involved in the determination of pesticide residues from the environmental waters. ECD – electron capture detector; NPD – nitrogen-phosphorus detector; TCD – thermal conductivity detector; FID – flame ionization detector

Contamination of water by pesticides is an important issue in many regions, posing problems in the environmental, water management and health sectors. To assess the extent of contamination of water, effective and properly designed analytical methods having sufficient sensitivity and accuracy are needed.

The analysis of pesticides usually includes isolation of the chemicals from the environmental matrix, clean-up, and chromatographic separation and quantification of the analytes. The initial part of any of these methods comprises sampling and sample preservation and preparation. The method should be reliable, repeatable, and applicable for compounds with variable physical-chemical characteristics, which makes the development of universal methods a challenging task.

Figure 2.7 shows the steps and methods involved in the analysis of environmental water samples starting from sampling to the last step of chromatographic identification. Appropriate sampling method is essential to collect representative sample for the desired analysis purpose. After the sampling step, the samples have to be preserved and stored properly. Sample preparation is the crucial step in the analysis as sample extraction techniques, extract clean-up and/or preconcentration are those operations with probable sources of inaccuracy and imprecision that can inadvertently be introduced into the entire analytical procedure.

2.4.1 Sampling

The objective of sampling is to collect a portion of material small enough in volume to be transported conveniently and handled in the laboratory while still accurately representing the material being sampled (Clesceri *et al.*, 1989).

The sample obtained during the collection phase should not deteriorate or become contaminated before arriving at the laboratory. Any of the components of interest that are present in the sample are at parts per million concentration levels and even lower. It is possible that these may alter completely or partially if the collection is faulty, or if the person who collects the sample doesn't take the necessary precautions for conservation. The collection should be carried out carefully with the purpose of guaranteeing an analytical result that will faithfully represent the real composition (Meyers, 2000).

Representative samples of some sources can be obtained only by making composites of samples collected over a period of time or at many different sampling points. Sometimes it is more informative to analyze numerous separate samples instead of one composite so as not to obscure maxima and minima (Clesceri *et al.*, 1989).

2.4.1.1 Sampling site and sample volume

The sampling site must be selected with respect to the objectives of a monitoring program or a survey. Thus, the overall monitoring strategy predetermines the choice of profiles in water streams or the number and/or the depth of the monitoring wells. For instance, if the objective is to monitor transboundary pollution of the water stream, the location should be as close as possible to the border. If the objective is to assess the quality of water used for drinking-water production, the sampling site should be positioned close to the intake. The

number and location of sampling stations for ground-water monitoring is a function of the objectives and scale of assessment (background or trend monitoring, emergency surveys around a spill, operational surveillance of the quality of potable water resources), the hydrogeological complexity and economic consideration (Meyers, 2000).

In all those cases it must be assured that the location is suitable for taking representative samples with due regard for the objective of the sampling. Furthermore, to assure representativeness of the sample, replicate samples should be taken occasionally to determine temporal and spatial variability. Sample volume depends on the number of analyses to be performed and on the technical requirements of a particular analysis. The volume of the water sample required for a single analysis of pesticides usually ranges from tens of milliliters up to 1 – 2 L, depending on the methodology applied (Meyers, 2000).

2.4.1.2 Sampling method

In general, the type of sampling depends on the goal of the monitoring program and differs to for river water, reservoir, groundwater, rainwater, wastewater, drinking water or pore water from the unsaturated zone. In river waters the sampling methods are usually based on bottle collection or water pump systems. When a homogeneous reach of a stream is monitored, the collection of samples in a single vertical mode may be sufficient. For small streams a grab sample taken at the centroid of flow is usually adequate (Meyers, 2000).

Even for one particular matrix the type of sampling can vary depending on the character of the information that is to be obtained from the monitoring. There are principally two types of samples: grab samples and composite samples. A grab sample is taken at a selected location and time, and then analyzed for pesticides. The collection of grab sample is

appropriate when it is desired (i) to characterize water quality at a particular time and location, (ii) to provide information about minima and maxima and (iii) to analyze parameters which can be subject to change. A composite sample is obtained by mixing several discrete samples of equal or weighted volumes collected at regular time intervals in one container, which is subsequently analyzed for the parameters of interest (Meyers, 2000).

The selection of an appropriate sampling strategy has a considerable effect on the information output from a monitoring campaign. To ensure that the collected water sample is a real representative of the sampled site and to avoid the detection of false positives, strict quality-control principles should be followed. These principles include qualification of sampling staff, checking for purity of sampling containers and collecting field blanks and field check samples. The use of duplicate samples for checking of sample stability and for eliminating random sampling errors is highly advisable (Meyers, 2000).

2.4.1.3 Preservation of water samples

Preservation of the sample during transport and storage depends on the type of pesticides to be analyzed. The storage of the water sample at 4°C, minimization of the volume of the gaseous phase in the container and the use of gas-tight caps is recommended. Hydrophobic pesticides can be easily adsorbed on polymer surfaces. From an aqueous sample containing highly lipophilic organic compounds that are stored in a common plastic bottle, more than 90% of these compounds can be adsorbed within 24 hour. Therefore, the storage of water samples in plastic containers must be avoided and the use of glass vessels only is recommended.

Many modern polar pesticides can easily hydrolyse when the pH reaches a certain critical value. Hence it is necessary to maintain the pH at a desired value using a buffer solution or, usually in the case acidic compounds, simply by acidifying the water sample. Keeping the samples in the dark and using amber-glass sample containers when available should prevent photolysis of the analytes. The presence of bioorganisms in water leads to biodegradation of dissolved pesticides. To suppress the biological activity of the aqueous environment, biodegradation inhibitors are used (Meyers, 2000).

2.4.2 Extraction methods

In general, environmental waters cannot be analyzed without sample pretreatment because they are too dilute or too complex. Sample preparation is the series of steps required to transform a sample so that it is suitable for chromatographic analysis. Sample preparation could include dissolving the sample, extracting analyte from a complex matrix, concentrating a dilute analyte to a level that can be measured, chemically converting analyte to a detectable form, and removing or masking interfering species (Harris, 2002). Current methods of analysis for aqueous samples involve liquid-liquid extraction (LLE), solid-phase extraction (SPE) and solid-phase microextraction (SPME).

2.4.2.1 Liquid-liquid extraction (LLE)

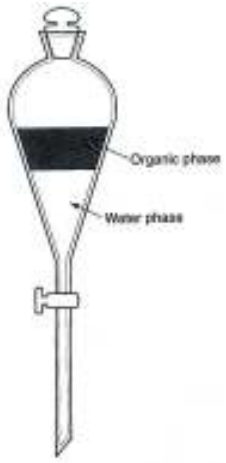


Figure 2.8: Separation funnel for liquid-liquid extraction method (Dean, 1998)

The most conventional and commonly used approach for the extraction of analytes from aqueous samples is liquid-liquid extraction (LLE). Analytes are extracted by the solvents from the environmental samples and/or further go through clean-up steps prior to instrumental analysis. The principal of LLE is that the sample is distributed or partitioned between two immiscible solvents in which the analyte and matrix have different solubilities. The main advantages of this approach are the wide availability of pure solvents and the use of low-cost apparatus (Dean, 1998).

Most of the classical preconcentration methods employing LLE have a relatively similar pattern: a water sample is extracted two or three times with a small volume (usually 10 – 50 mL) of an organic solvent (hexane, heptane, cyclohexane, methylene chloride, Freon, etc.) and the extracts (plus the solvent used for rinsing the internal surface of the glassware used for the extraction) are dried with purified sodium sulfate, filtered, evaporated either to dryness or to a volume usually under 1 mL and redissolved in a small volume of the solvent compatible with a selected chromatographic technique. To remove interfering

compounds, the extract can be cleaned by percolation through a column packed with Florisil, alumina or another suitable sorbent (Meyers, 2000).

The selectivity of LLE is dependent on the solvent used and the nature of the aqueous matrix. Other parameters which affect isolation of organic from water samples such as pH, ionic strength, water:solvent ratio, number of extractions and; type and concentration of analyte must be considered (Chee *et al.,* 1993). However, although LLE appears simple and does not require complex equipment, it is laborious, time consuming and expensive, and subjected to practical problems such as emulsification, the use and disposal of large volumes of highly pure and possibly toxic organic solvents, slow evaporation steps, and the risk of loss and contamination (Chee *et al.*, 1993).

2.4.2.2 Solid-phase extraction (SPE)

(a) (b) (c)

Figure 2.9: **(a)**: SPE using a cartridge and a single side arm flask apparatus; **(b)**: vacuum manifold for SPE of multiple cartridges; and **(c)**: SPE using an SPE disk and a single side arm flask apparatus. (Dean, 1998)

Solid-phase extraction, which has been developed intensively in recent decades, has become a powerful alternative technique to LLE owing to its simplicity, flexibility and high sample throughput (Chee, *et. al*., 1993; Meyers, 2000). Additional advantages include reduction of toxic solvent consumption and greater health safety (Meyers, 2000).

SPE is widely used for trace analysis and determination of micropollutans, consists of passing a liquid sample (pure or solution) over a solid sorbent. Figure 2.10 shows a frequently encountered situation. In this example, the analyte is the only compound retained by the sorbent-containing column. Other substances are eliminated by rinsing the column after the analyte has been adsorbed. Following rinsing, the compound of interest is desorbed using an appropriate solvent. This extraction procedure allows not only isolation of the analyte but also its preconcentration (Rouessac and Rouessac, 2000).

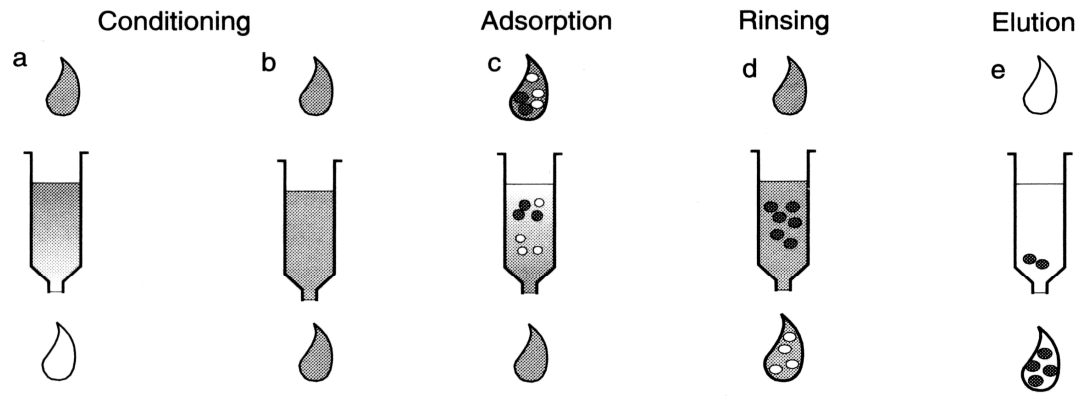


Figure 2.10: Steps involved in solid-phase extraction (SPE). (a) activation of the sorbent; (b) rinsing of the column; (c) introduction of the sample; (d) elimination of interferences by rinsing; (e) desorption of the analyte. (Dean, 1998)

Many factors influence the efficiency of the SPE process, the two most important being capacity and retention. An insufficient capacity of the sorbent surface can cause its overloading and, consequently, earlier breakthrough of analytes. The more critical factor is the retention of analytes, which should be a maximum in the water-sorbent-analyte system and a minimum in the eluent-sorbent-analyte system. The existence of these two contradictory demands on the strength of the sorbent-analyte interactions leads to the necessity to make a compromise during the selection of working conditions for sorption and desorption so as to obtain an optimum preconcentration (Meyers, 2000).

Today, cartridge-based SPE has blossomed into a widely practiced technique as a wider variety of solid-phase supports becomes available. Sorbents such as carbon, alumina, silica, porous polymers, C_8, C_{18}, aminopropyl-silica, cyano and Florisil are either self-packed or commercially prepared in disposable cartridges under trade names such as Supelclean, Quick-Sep, Sep-Pak and Bond-Elut are available in the market. Cartridges for SPE are useful for field sampling and reduce sample manipulation, solvent consumption and labour cost by allowing batches of 12 – 24 samples to be prepared simultaneously, without any risk of sample contamination (Chee *et al.*, 1993).

2.4.2.3 Solid-phase microextraction (SPME)

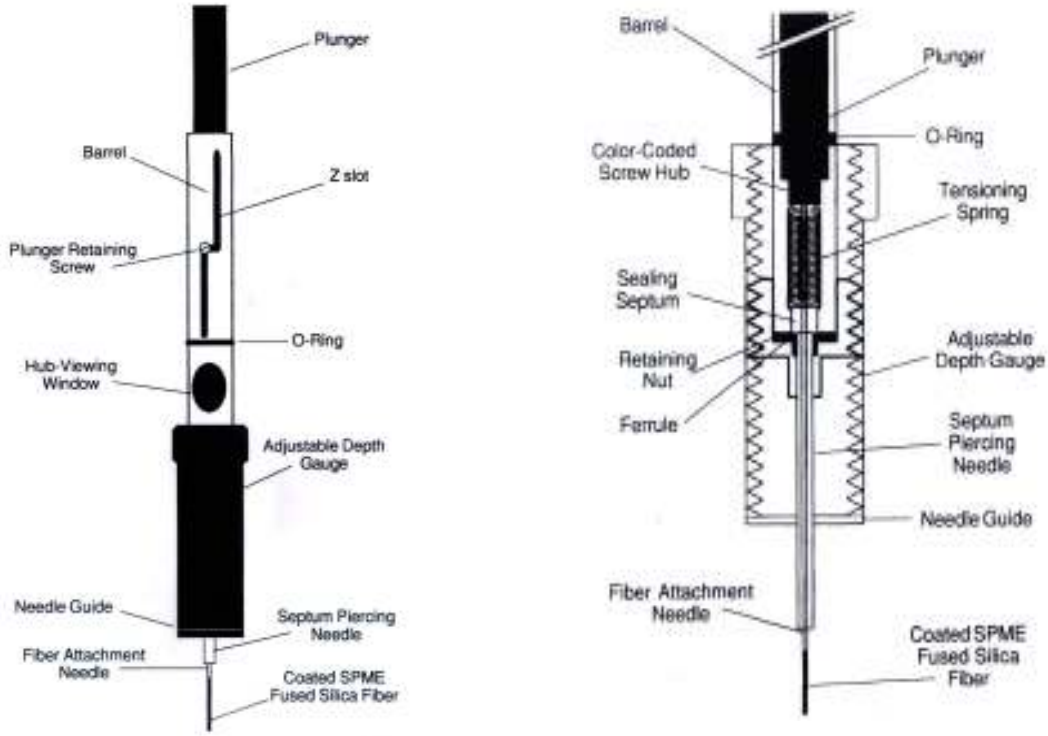


Figure 2.11: SPME holder and section view. (Dean, 1998)

Solid-phase microextraction or SPME was developed to address the need for fast, solvent-free and field-compatible sample preparation technologies. SPME was introduced as a solvent-free sample preparation technique in 1990. The basic principle of this approach is to use a small amount of the extracting phase, usually less than one microliter. The sample volume can be very large, when the investigated system, for example air in room or lake water, is sampled directly. The extracting phase can be either a high-molecular-weight polymeric liquid, similar in nature to stationary phases in chromatography, or it can be a solid sorbent, typically of a high porosity to increase the surface area available for adsorption (Meyers, 2000).

To date the most practical geometric configuration of SPME utilizes a small fused-silica fiber, usually coated with a polymeric phase. The fiber is mounted for protection in a syringe-like device (Figure 2.11). The analytes are absorbed or adsorbed by the fiber phase (depending on the nature of the coating) until an equilibrium is reached in the system. The amount of an analyte extracted by the coating at equilibrium is determined by the magnitude of the partition coefficient (distribution ratio) of the analyte between the sample matrix and the coating material (Meyers, 2000).

SPME is the process whereby an analyte is adsorbed onto the surface of a coated-silica fiber as method of concentration. This is followed by desorption of the analytes into a suitable instrument for separation and quantitation. Development of a particular procedure for determination of pesticides in water samples using the SPME technique usually requires the optimization of the variables related to both extraction and desorption steps. There are several variables studied including almost inevitably fiber type (Magdic *et al.*, 1996; Lambropoulou *et al.,* 2000; Sampedro *et al.,* 2000; Lambropoulou and Albanis, 2001; Lambropoulou *et al.*, 2002), extraction time (Valor *et al*., 1997; Aguilar *et al*., 1998; Lambropoulou and Albanis, 2001; Lambropoulou *et al.,* 2002), extraction temperature (Valor *et al*., 1997; Aguilar *et al*., 1998; Lambropoulou and Albanis, 2001;), pH adjustment (Magdic *et al.*, 1996; Aguilar *et al*., 1998; Sampedro *et al.,* 2000; Lambropoulou *et al.,* 2002) and ionic strength (Magdic *et al.*, 1996; Aguilar *et al*., 1998; Lambropoulou *et al.,* 2000; Sampedro *et al.,* 2000; Lambropoulou and Albanis, 2001; Lambropoulou *et al.*, 2002) for the extraction step; and temperature (Aguilar *et al*., 1998; Sampedro *et al.,* 2000) and time (Aguilar *et al*., 1998) in the desorption step (Magdic *et al.*, 1996; Valor *et al.*, 1997; Aguilar *et al.*, 1998; Lambropoulou *et al.*, 2000; Sampedro *et al.*, 2000; Lambropoulou and Albanis, 2001; Lambropoulou *et al.*, 2002). The extraction of a

sample by SPME is usually conducted directly with the coating fiber immersed in the liquid phase of the sample. Headspace SPME where the fiber is exposed to the sample headspace to extract target analytes is another development of the technique. Headspace approach is preferred when the sample matrix contains undissolved particles or non-volatile material which may contaminate or damage the coated fiber.

2.4.3 Instrumentation: Gas chromatography

After a series of sample preparation steps, the analysis of pesticide residues from environmental sample will be culminated in the use of chromatographic separation coupled with a suitable detector. Gas chromatography (GC) using capillary columns and selective detection systems is the preferred analytical technique because of its high resolution, speed of analysis and low cost.

Gas chromatography (sometimes called gas-liquid chromatography or GLC) has developed from a single successful application for separating volatile carboxylic acids in the early 1950s into a universally accepted chemical measurement tool spanning the disciplines of chemistry, biochemistry, forensics, toxicology, environmental sciences, and others. The development of GC arose in the context of surging interests in electronics and analytical instrumentation during the post-World War II era (Meyers, 2000).

A growing reliance then and now upon physical methods for chemical analyses, such as mass spectrometry (MS) or infrared spectrometry, meant that instrumental characterizations of complex mixtures would be difficult to interpret without prefractionating a sample into individual constituents. GC met this requirement for volatile and semivolatile organic compounds. These compounds constitute only a fraction

of all organic substances; however, their relative importance in foodstuffs, cosmetics, and medicines, or in some instances as persistent and toxic pollutants in the environment, guaranteed a role for GC in modern analytical methods (Meyers, 2000).

In gas chromatography, a mobile phase (a carrier gas) and a stationary phase (column packing or capillary column coating) are used to separate individual compounds. The carrier gas can be nitrogen, argon-methane, helium, or hydrogen. The column is installed in an oven with the inlet attached to a heated injector block and the outlet attached to a detector. Precise and constant temperature control of the injector block, oven, and detector is maintained. Stationary-phase material and concentration, column length and diameter, oven temperature, carrier gas flow, and detector type are the controlled variable. A schematic diagram of a gas chromatograph is given in Figure 2.12.

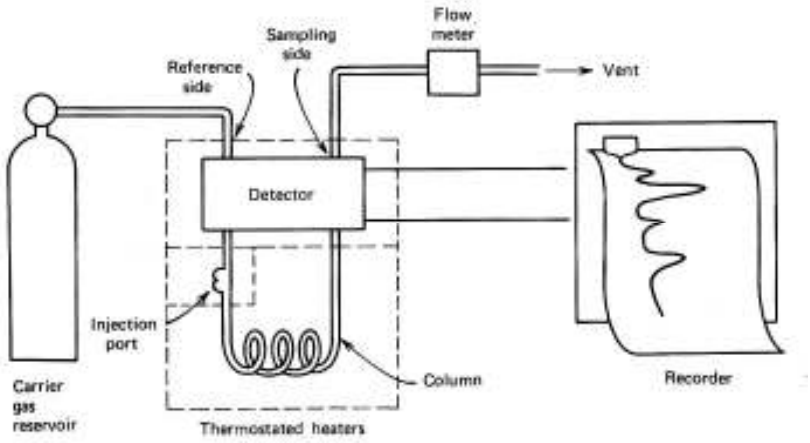


Figure 2.12: Schematic diagram of a gas chromatograph (Meyers, 2000)

2.4.3.1 Principles of gas chromatography

In a GC experiment, a vapor sample is moved with a flowing gas (the mobile phase, generally either nitrogen or helium) through a glass or metal column containing a phase that is immobilized (the stationary phase). The stationary phase is typically a low-vapor-pressure liquid polymer, and is either coated or chemically bonded to a stationary support (either an inert solid or simply the inner wall surface of an open tube, i.e. a capillary column) (Meyers, 2000).

As the mobile or gas phase is forced, under pressure, through the column, the sample components are also carried toward the detector at a speed dependent upon the chemical structure of the sample components, the characteristics of the stationary phase, the column temperature, and specifics of the column such as gas flow rate and the amount of stationary phase. Differences in the time of passage through the column (the elution time) are described by the extent to which a substance is dissolved into the stationary phase, i.e. the partition coefficient K or the ratio of concentrations in the stationary and mobile phases. Thus, the separation process is founded in differences in the partition or solubility of various analytes in the stationary phase. It is the time spent in the stationary phase that prescribes the retention volume; all compounds spend the same amount of time in the mobile phase (Meyers, 2000).

2.4.3.2 Gas chromatography columns

The gas chromatographic column is the central item in a gas chromatograph. Over the last three decades the nature and design of the column has changed considerably from one containing either a solid adsorbent or a liquid deposited on an inert solid support packed into a length of tubing to one containing an immobilized or cross-linked stationary phase

bound to the inner surface of a much longer length of fused silica tubing. Column tubing fabricated from copper, aluminum, glass, and stainless steel served the early analytical needs of gas chromatographers (Grob, 1995).

Capillary Columns

The introduction of inert fused-silica capillary columns in 1979 markedly changed the practice of gas chromatography, enabling high-resolution separations to be performed in most laboratories (Grob, 1995). After 1979 the use of packed columns began to decline (Grob, 1995). The capillary column, also referred to as an open tubular column because of its open flow path, offers a number of advantages over the packed column. These merits include vastly improved separations with higher resolution, reduced time of analysis, smaller sample size requirements, and often higher sensitivities.

Capillary columns are usually prepared from high purity fused silica obtained by the combustion of SiH_4 (or $SiCl_4$) in an oxygen-rich atmosphere. The internal diameter varies from 0.1 – 0.35 mm and the length from 15 to 100 m. Capillary columns are usually coated on the outside polyimide or a thin aluminum film. Polyimide mechanically and chemically protects the column (maximum temperature = 370˚C). The columns are coiled around a lightweight, metallic support. The internal surface of the silica is usually treated or silanized, depending on the technique used to bond the stationary phase. For wall-coated open tubular (WCOT) columns, the stationary phase covers the inside surface of the column. The film thickness of the stationary phase can vary from 0.05 to 5 μm. It can be simply deposited on the surface, can originate from the reticulation of a polymer on the silica surface or can be bound to the silica through covalent bonds (Rouessac and Rouessac, 2000).

Packed Columns

Packed columns, less commonly used today, are made of stainless steel or glass. They have diameters of 1/8 or 1/4 in (3.18 or 6.35 mm) and range in length from 1 to 3 m. The internal surface of the tube is treated to avoid catalytic interactions with the sample. These columns use a carrier gas flow rate of typically 10 to 40 ml/min. Although they are still used in approximately 10% of cases for routine GC work, packed columns are not well adapted to trace analyses. Packed columns contain an inert and stable porous support on which the stationary phase can be impregnated or bound (varying between 3 to 25%). The solid support is made of spheres of approximately 0.2 mm in diameter, obtained from diatomites, silicate fossils such as kieselguhr, Tripoli whose skeleton is chemically comparable to amorphous silica (Rouessac and Rouessac, 2000).

2.4.3.3 Gas chromatography detectors

The subject of detectors in GC is a pivotal theme since the separation processes will have been wasted if the analyte cannot be detected. Effluent from the column enters a detector where the composition of the carrier gas stream is characterized through one of several possible chemical or physical properties of molecules. The mainstays in GC have been the flame ionization detector (FID), the thermal conductivity detector (TCD) and the electron capture detector (ECD). Other commercially available detectors include the flame photometric detector (FPD) and the nitrogen-phosphorus detector. Additional techniques such as mass spectrometry are used to assist in identification of the eluted components.

Flame Ionization Detector (FID)

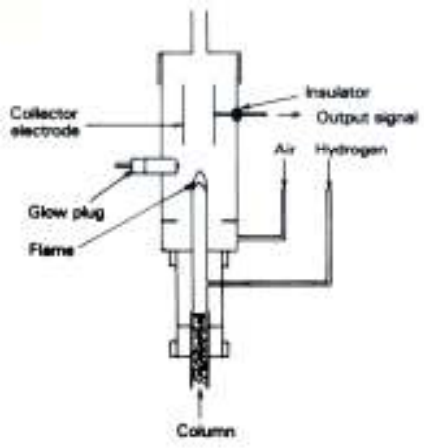


Figure 2.13: Flame ionization detector (Christian, 1994)

Most organic compounds form ions in a flame. This forms the basis of an extremely sensitive detector, the flame ionization detector (Christian, 1994). This detector, considered to be universal for the analysis of organic compounds, appears ideal for gas chromatography. The gas flow exiting the column passes through a small burner fed by hydrogen air. This detector essentially destroys the sample. Combustion of the organic compounds flowing through the flame creates charged particles that are responsible for generating a small current between two electrodes (voltage differential of 100 – 300 V) (Rouessac and Rouessac, 2000). The signal is amplified and conditioned by an electrometer amplifier enabling a chart recorder, integrator or computer interface to be easily used to produce the chromatogram and data. Materials not detected by the FID include H_2, O_2, N_2, $SiCl_4$, SiF_4, H_2S, SO_2, COS, CS_2, NH_3, NO, NO_2, N_2O, CO, CO_2, H_2O, Ar, Kr, Ne, Xe; HCHO and HCOOH have a very small response (Braithwaite and Smith, 1996).

Thermal Conductivity Detector (TCD)

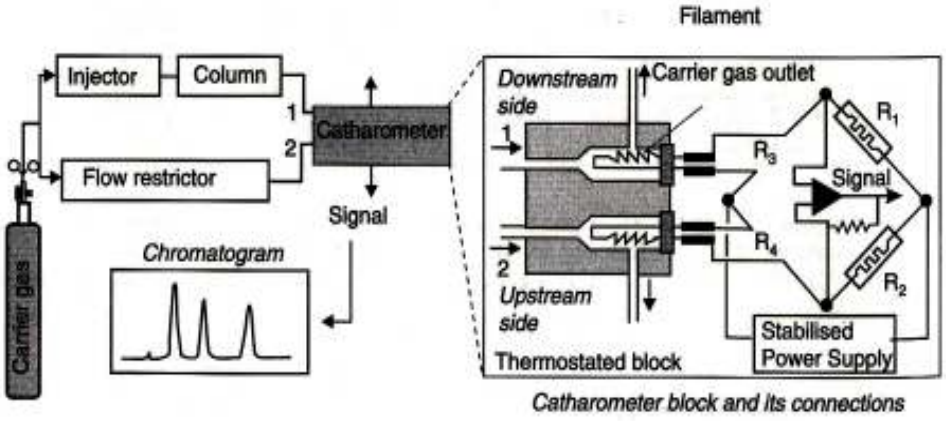


Figure 2.14: Thermal conductivity detector. To the left is a schematic showing the path of the carrier gas. To the right is a schematic of the TCD and its operating principle, based on an electrical Wheatstone bridge (equilibrium exists when $R_1/R_2 = R_3/R_4$). (Rouessac and Rouessac, 2000)

The thermal conductivity detector (TCD) is one of the most commonly used detectors in gas chromatography. It measures changes in the thermal conductivity of the carrier gas caused by the presence of eluted substances. The thermal conductivity affects the resistance of the thermistor as a function of the temperature.

The detector incorporates two identical thermistors, resembling minuscule filaments, which are placed inside a metallic block held at a temperature above that of the column. One of the filaments is flushed by the carrier gas re-routed prior to the injector while the other is flushed by the carrier gas exiting the column. In the steady state, a temperature equilibrium exists, which depends on the resistance and which in turn is a function of the

thermal conductivity of the gas and of the electrical current flowing through the filament. When a solute elutes from the column, there is a change in the composition of the mobile phase and thus in the thermal conductivity. This results in a deviation from thermal equilibrium, causing a variation in the resistance of one of the filaments. This variation is proportional to the concentration of analyte, provided its concentration in the mobile phase is low (Rouessac and Rouessac, 2000).

The TCD responds to all types of organic and inorganic compounds including those not detected by the FID. It does not destroy the eluted components and therefore is suitable for use with fraction collectors for trapping of the separated components for preparative work (Braithwaite and Smith, 1996).

Electron Capture Detector (ECD)

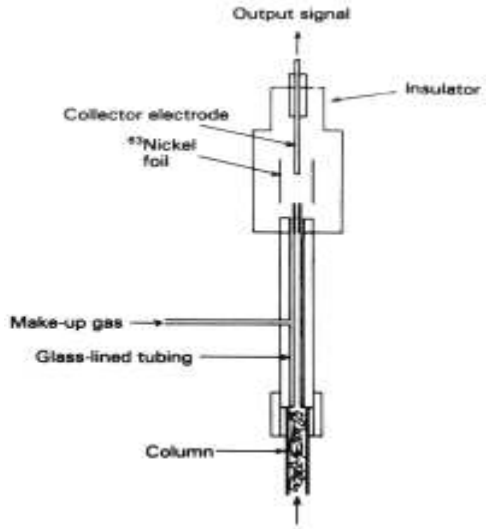


Figure 2.15: Electron capture detector (Braithwaite and Smith, 1996)

The electron capture detector (ECD) usually is used for the analysis of compounds that have high electron affinities, such as chlorinated pesticides, drugs, and their metabolites.

This detector is somewhat selective in its response, being highly sensitive toward molecules containing electronegative groups: halogens, peroxides, quinones, and nitro groups. It is insensitive toward functional groups, such as amines, alcohols, and hydrocarbons (Clesceri *et al.*, 1998). The ECD responds to changes in electrical conductivity of gases in an ionization chamber due to the presence of electron acceptor molecules (Braithwaite and Smith, 1996).

The detector is operated by passing the effluent from the gas chromatographic column over a radioactive beta particle emitter, usually nickel-63 or tritium adsorbed on platinum or titanium foil. An electron from the emitter ionizes the carrier gas, preferably nitrogen, and produces a burst of electrons. About 100 secondary electrons are produced for each initial beta particle. After further collisions, the energy of these electrons is reduced to the thermal level and they can be captured by electrophilic sample molecules (Clesceri, *et. al.*, 1998).

The electron population in the ECD cell is collected periodically by applying a short voltage pulse to the cell electrodes and the resulting current is compared with a reference current. The pulse interval is adjusted automatically to keep the cell current constant, even when some of the electrons are being captured by the sample. The change in the pulse rate when a sample enters the ECD is then related to the sample concentration. The ECD offers linearity in the range of 10^4 and subpicogram detection limits for compounds with high electron affinites (Clesceri *et al.*, 1998).

Nitrogen-phosphorus Detector (NPD

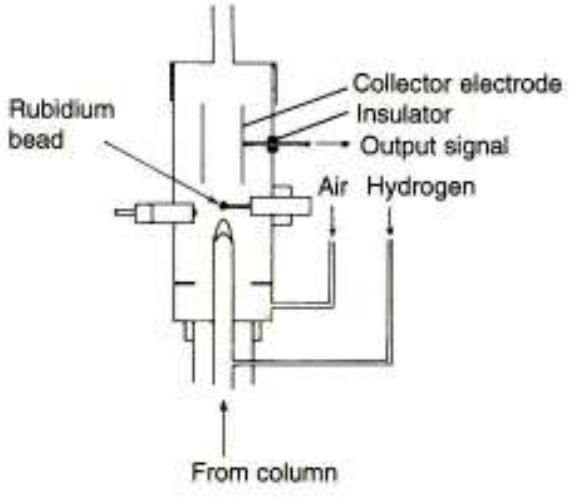


Figure 2.16: Nitrogen-phosphorus detector (Grob, 1995)

Today's nitrogen-phosphorus detector (NPD) evolved from an earlier type of gas chromatographic detector known as the alkali flame ionization detector (AFID). The nitrogen-phosphorus detector also known as thermoionic detector (TID) (Grob, 1995). This thermoionic detector is very sensitive to compounds that contain nitrogen or phosphorous. It operates in a different mode from the FID detector.

The NPD detector incorporates, between the flame and collector, a piece of ceramic doped with an alkaline salt (Rb or Cs). Due to the catalytic effect of the alkaline salt, compounds containing nitrogen or phosphorous produce more ions than other molecules. Nitrogen present in air does not, however, yield any signal. There are several types of NPD detector and, depending on the type; compounds are ionized in different ways. The flame used in these detectors is much cooler that that used in an FID and an electrical current is used to heat the ceramic, producing an alkaline plasma necessary for the operation of this detector (Rouessac and Rouessac, 2000).

Flame Photometric Detector (FPD)

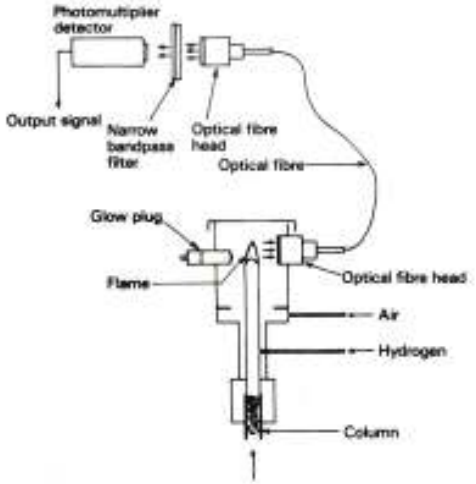


Figure 2.17: Flame photometric detector (Grob, 1995)

The flame photometric detector is specific for compounds containing sulphur or phosphorous. Compounds eluting from the column are burned in a flame hot enough to excite these elements and induce photonic emission, which is detected by a photomultiplier. Optical filters are used in the detection system to monitor wavelengths that are characteristic of these substances (Rouessac and Rouessac, 2000).

Mass Spectrometry (MS)

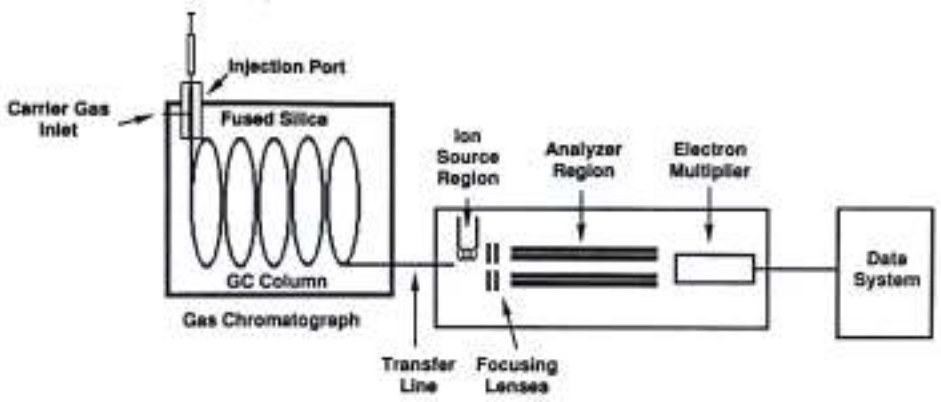


Figure 2.18: Schematic diagram of a typical gas chromatograph/mass spectrometer system. (Grob, 1995)

Mass spectrometry (MS) is an analytical method based on the determination of atomic or molecular masses of individual species in a sample. Information acquired allows determination of the nature, composition, and even structure of the analyte.

The schematic diagram of a typical capillary GC/MS system is shown in Figure 2.18. The gaseous effluent from the chromatograph is directed through the transfer line into the ion source. The vaporized analytes are then ionized, producing molecular and/or fragment ions which are then mass resolved and detected. The resulting mass spectrum is displayed as a plot of the relative intensity of these ions versus their mass to charge ratio (*m/z*). Since most ions produced are singly charged, their *m/z* values are indicative of their masses (Grob, 1995).

2.5 Method validation/Quality assurance

Before a new analytical method or sample preparation technique is to be implemented, it must be validated. The various figures of merit need to be determined during the validation process. Random and systematic errors are measured in terms of precision and bias. The detection limit is established for each analyte. The accuracy and precision are determined at the concentration range where the method is to be used. The linear dynamic range is established and the calibration sensitivity is measured. In general, method validation provides a comprehensive picture of the merits of a new method and provides a basis for comparison with existing methods. A typical validation process involves one or more of the following steps (Mitra, 2003):

- *Determination of the single operator figures of merit*. Accuracy, precision, detection limits, linear dynamic range, and sensitivity are determined. Analysis is performed at different concentrations using standards.
- *Analysis of unknown samples*. This step involves the analysis of samples whose concentrations are unknown. Both qualitative and quantitative measurements should be performed. Reliable unknown samples are obtained from commercial sources or governmental agencies as certified reference materials. The accuracy and precision are determined.
- *Equivalency testing*. Once the method has been developed, it is compared to similar existing methods. Statistical tests are used to determine if the new and established methods give equivalent results. Typical tests include Student's *t*-test for a comparison of the means and the *F*-test for a comparison of variances.

- *Collaborative testing*. Once the method has been validated in one laboratory, it may be subjected to collaborative testing. Here, identical test samples and operating procedures are distributed to several laboratories. The results are analyzed statistically to determine bias and interlaboratory variability. This step determines the ruggedness of the method.

CHAPTER 3 – MATERIALS AND METHODS

3.1 SPME fiber

The SPME was performed with commercially available 100µm poly(dimethylsiloxane) (PDMS) coated fiber and housed in the appropriate manual holder. The SPME fibers and holder were purchased from Supelco, USA. The PDMS fiber was conditioned before initial application in the hot port of the gas chromatograph by heating it at 250 ºC for 1 h according to manufacturer's instruction.

3.2 Chemicals and reagents

Pestanal grade pesticides (azinphos ethyl, chlorpyrifos methyl, diazinon, dichlorvos, endosulfan I, endosulfan II, endosulfan sulfate and malathion) from Riedel-de Haën were purchased from Sigma Aldrich, USA. Degrees of purity were > 95% for all pesticides. For the preparation of standard stock solutions, Milipore filtered (by 0.45µm membrane filter paper) methanol AR grade from Fisher Scientific, USA was used. Working solutions of pesticides were prepared daily from Milipore filtered distilled water. The concentration of individual pesticide in the mixed standard solution was prepared by mixing 20 ppb of chlorpyrifos methyl, diazinon, endosulfan I, endosulfan II, endosulfan sulfate, malation and 400 ppb of azinphos ethyl, dichlorvos.

Acetic acid (CH_3CO_2H), sodium acetate (CH_3CO_2Na), potassium phosphate monobasic (KH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), sodium bicarbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3), and distilled water were used to prepare buffer during the optimization of the pH adjustment parameter.

Sodium chloride (NaCl) and distilled water were used to adjust the ionic strength of the sample as well as standard during the ionic strength parameter optimization.

3.3 Equipment and instrumentation

3.3.1 Sampling

A four (4) seated fiber boat with a petrol engine was utilized during the sampling expedition, each of the passengers was equipped with a set of survival suit for safety purpose. A Trex Legend portable global positioning system (GPS) from GARMINe was used to determine the sampling location when navigating along the river.

In-situ water sample parameters: pH, temperature, dissolved oxygen and salinity were measured by pH meter cum thermometer from Hanna; DO meter from Hanna; and salinity refractometer from Hanna. The parameters measured were then recorded in a log book. A digital camera was utilized for the data recording purpose as well.

The water samples were collected into the labeled 1-Litre sampling bottles with screwed-cap from Schott Duran, Germany and then kept in an ice chest at about 4°C during the transportation. The water samples were then filtered through 0.45µm filter paper (Schleicher & Schuell, Germany) and a Milipore filter equipped with a portable hand-pump before transported to the laboratory.

3.3.2 Solid-phase microextraction

The samples were transferred into a 15ml screw-cap vials supplied with PTFE/silicone septa (Supelco, USA) during the parameters optimization as well as quantitative

determination. Samples were stirred throughout the whole SPME parameters optimization process aided with a ceramic topped digital stirring hotplate (Fisher Scientific, Japan), 10mm x 0.4mm PTFE coated stir bars and a countdown/up digital timer. A water bath was utilized to control the sample temperature to a constant level.

3.3.3 Gas chromatographic analysis

Gas chromatographic analysis was perfomed using a Shimadzu GC – 17A, fitted with an ECD (Fisher Scientific, Japan). SPME GC column inlet for Shimadzu 17A with splitless injector (length 95 mm x O.D. 5 mm x I.D. 0.75 mm) (Fisher Scientific, Japan) and Thermogreen LB-2 septum Shimadzu plugs (Fisher Scientific, Japan) was utilized for the SPME fiber injection. Separations were conducted using a Supelco BPX-5, 30m x 0.25 mm ID; with 0.25μm of film thickness column.

The data produced from the GC was transferred to the computer via Shimadzu® CBM-102 Communications Bus Module (Shidmazu, Japan) and then recorded and interpreted by the Shimadzu GCsolution Chromatography Data System Version 2.2 software (Shimadzu Corperation, Analytical and Measuring Instrument Division, Japan).

3.4 Methods

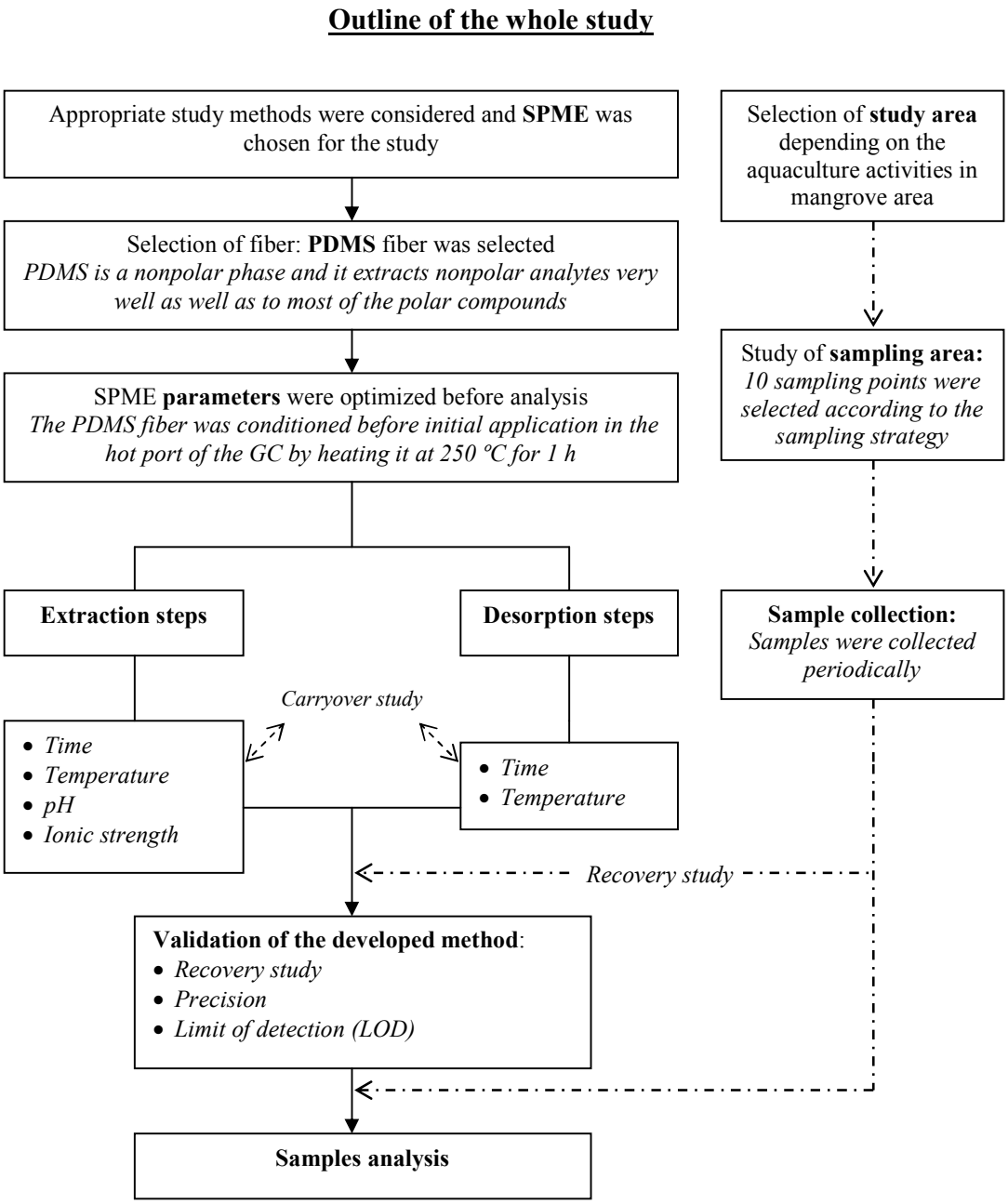


Figure 3.1: The schematic outline of the whole study from sampling to sample analysis as well as design of the analysis method.

3.4.1 Sampling

3.4.1.1 Study area

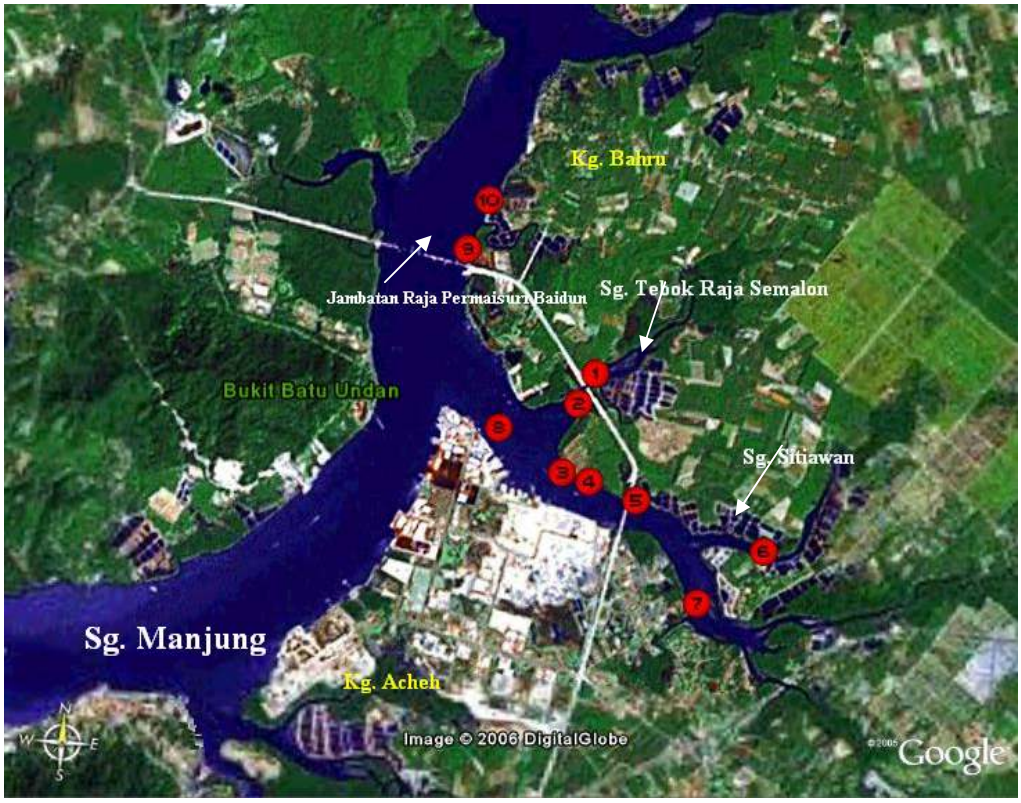


Figure 3.2: Location of the 10 sampling points positioned by the Global Positioning System (GPS) and the map is obtained from Google Earth program from the internet.

The study area included Sg. Manjung (previously known as Sg. Dinding), Sg. Tebok Raja Semalon and Sg. Sitiawan, located in Manjung, a district of Perak state, West Malaysia. Sg. Tebok Raja Semalon and Sg. Sitiawan are the tributaries of Sg. Manjung. Shrimp aquaculture activities are common in this study area whereby several linearly arranged earthen ponds of shrimp farms can be found along the river banks in the mangrove forest when navigating along the rivers. *P. monodon* is the most popular shrimp species commercially farmed around this area.

The sampling stations covered from the area of latitude (04º13'N to 04º18'N) and longitude (100º38'E to 100º42'E). Due to the inaccessibility into the shrimp farms, sample collections were done by collecting the water samples as near as to the discharges of the shrimp farms. Figures 3.3 – 3.6 are pictures taken at some sampling stations. Cage aquaculture can also be found when navigating along the rivers.

Figure 3.3: A view of shrimp farm. Heavy aeration of pond water is necessary in shrimp farming.

Figure 3.4: The picture showed the water intake and drainage system of the shrimp farms.

Figure 3.5: A closer view of the water intake and drainage system for the shrimp farms.

Figure 3.6: Cage aquaculture around the shrimp farms.

3.4.1.2 Sampling method

Three (3) periodical sampling expeditions were launched throughout the study period to monitor the water quality of the sampling area. During the first expedition, 10 sampling points were selected as close as to the water intake and drainage system of the shrimp farms due to the inaccessibility of the shrimp farms. A global positioning system (GPS) was utilized to locate the sampling points. For the second and third sampling expeditions, GPS was utilized to locate back the sampling points to ensure the repeatability of the samplings.

Water samples were collected by directly immersing the container beneath the water surface to a depth of one (1) foot (~ 30.5 cm). The sample containers were rinsed two to three times before collecting the sample. Duplicate samples were collected as close as possible to the same point in space and time and are intended to be identical. Field blanks consist of distilled water that is taken to the field and poured into the sample container. Field blanks are used to assess the contamination from field sources such as airborne materials, containers, and preservatives. The collected river water samples were kept in an ice chest during transportation to the laboratory for further sample treatment. The collected samples were then filtered through a Milipore filter utilizing 0.45 μm membrane filter paper and kept in chiller at 4˚C prior to analysis. The field blanks were treated in the same manner as the samples.

Parameters of the rivers were measured *in-situ* when collecting the water samples. Temperature, pH, dissolved oxygen and salinity of the water were recorded in a log book.

3.4.2 Optimization of SPME parameters

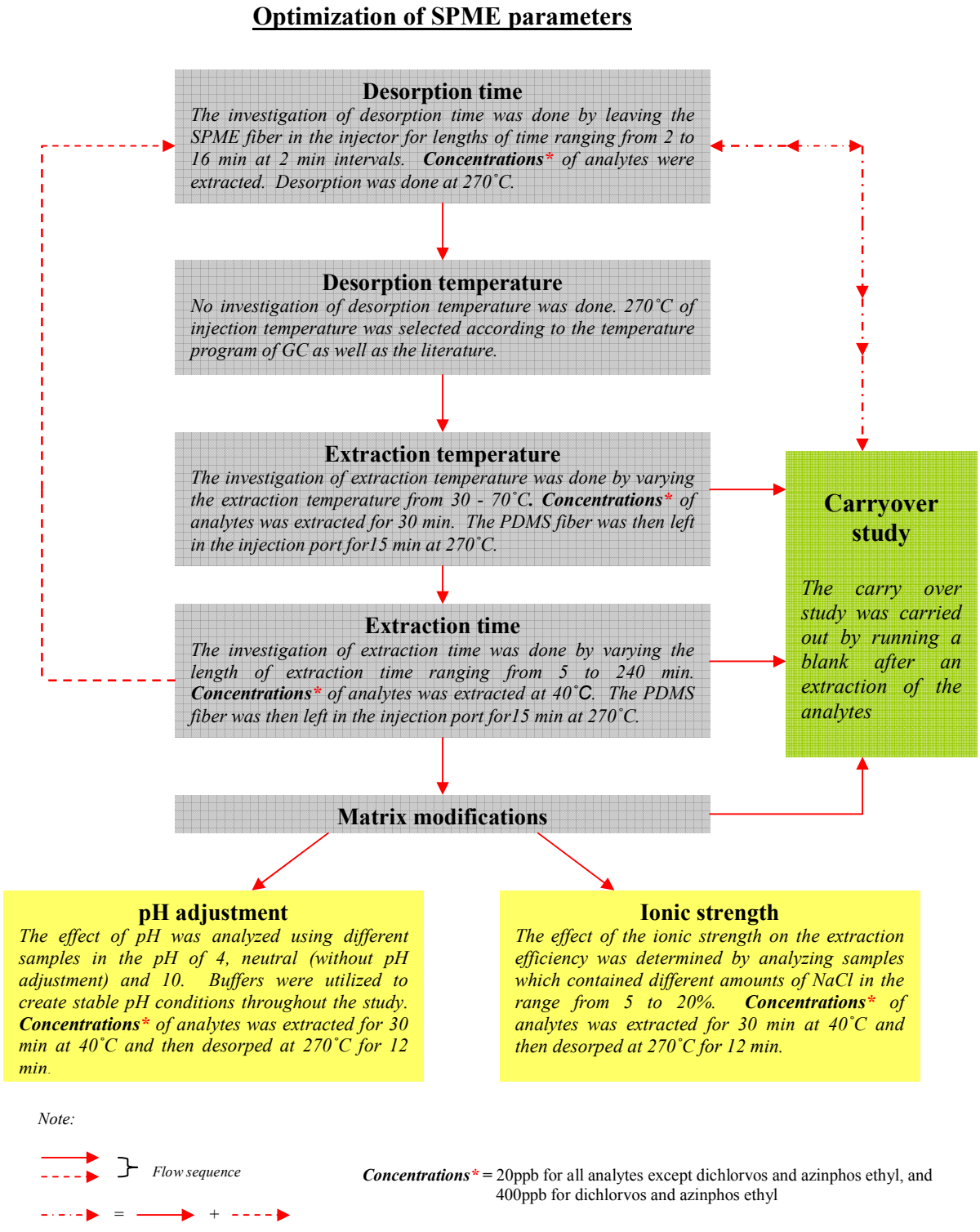


Figure 3.7: Flow chart of SPME parameters optimization steps.

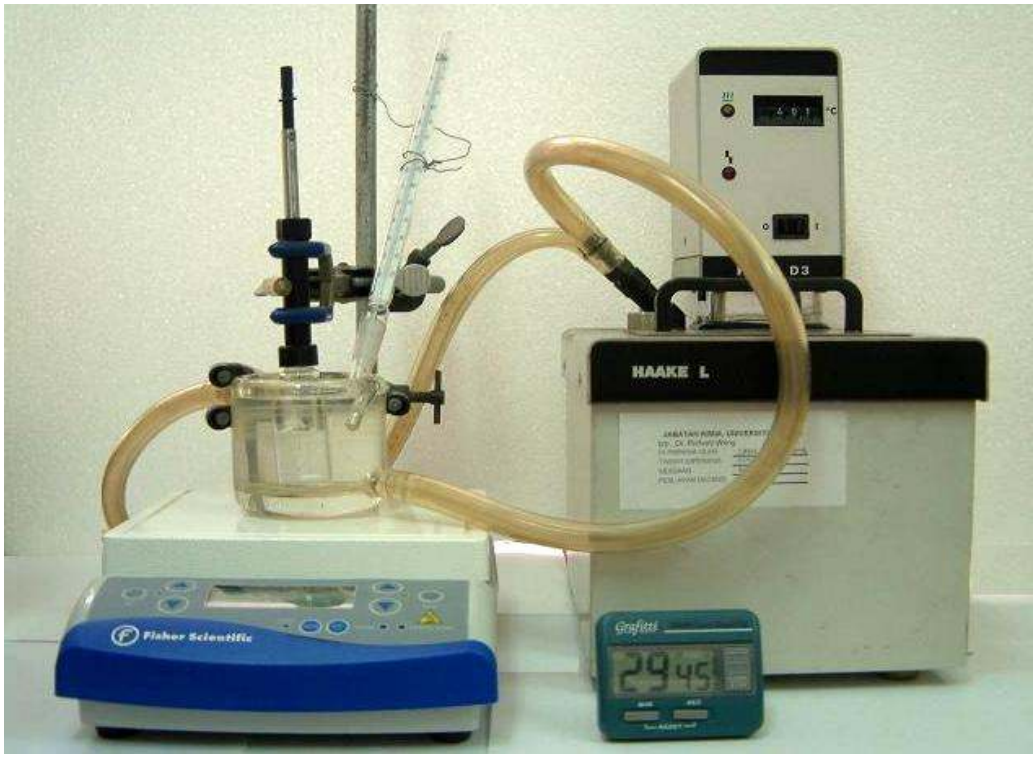


Figure 3.8: Solid-phase microextraction setup (SPME fiber and holder; digital magnetic stirrer; digital count up/down timer and water bath)

3.4.2.1 Extraction volume

10 ml of standard solution or sample was placed in 15 ml vials, sealed with hole-caps and PTFE line septa. A PTFE coated magnetic bar was put into the vial prior to the extractions for stirring purpose. The solution was stirred with a digital magnetic stirrer at 600 rpm (round per minute).

3.4.2.2 Desorption step and carryover study

Optimum desorption conditions were determined by testing the different lengths of time. The time and temperature required to successfully desorb all the analytes from the fiber

coating with minimal carryover in subsequent analysis (fiber blank) were considered to be optimized desorption conditions.

Desorption Time

The investigation of desorption time was done by leaving the SPME fiber in the injector for lengths of time ranging from 2 to 16 min at 2 min intervals. Mixed standard solutions were extracted under continuous stirring. Desorption was done at 270˚C. The investigation of desorption time was done to triplicate solutions.

After the desorption process, a blank will be carried out where another run of desorption of the fiber in the injector for 20 min. The purpose of doing the carryover study was to determine the completeness of the previous desorption. If traces of analytes was observed indicating that desorption time was not sufficient for the analytes to be totally desorped from the fiber to the injector.

Optimization of desorption time would be repeated whenever the extraction conditions were changed. Longer desorption time might be needed when the extraction efficiencies were improved as more analytes were extracted before reaching equilibrium.

Desorption Temperature

No investigation of desorption temperature was done. 270˚C of injection temperature was selected according to the temperature program of GC as well as from the literature.

3.4.2.3 Extraction steps

Extraction Temperature

Extraction temperature parameter was determined by maintaining the exposure time of the fiber to the mixed standard solutions for 30 min varying the temperature of water bath from 30 to 70°C. Triplicate of analyses were carry out. Continuous stirring 600 rpm of the aqueous solution was applied throughout the study. The analytes were then thermally desorped into the injection port of a gas chromatography at 270°C for 15 min.

Extraction Time

The fiber was systematically exposed to the mixed standard solutions for increasing time intervals in the ranges between 5 and 240 min. All the extractions were carried out in triplicates at 40°C under continuous stirring and the analytes were thermally desorped into the injection port of a gas chromatograph at 270°C for 15 min.

A count up/down digital timer was employed to ensure the absorption period is accurate to within ± 1 second. For example, in 30-minute extraction, the timer was set at 29 minutes 45 seconds, when countdowns to the preset time, the timer will further count-up for another 15 seconds. Once the 15^{th} second reached, the fiber would be withdrawn from the solution immediately. The countdown of 30 minute was set 15 second earlier as a purpose of reminder when the extraction was not attended to and the researcher would have enough time to reach the bench to stop the extraction, so that the extraction time will not exceeded the set period.

Carry over study was done again after every extraction, as the longer extraction time, the more analytes will be absorped onto the fiber, and the longer desorption time might be needed for the analytes to desorp from the fiber.

Matrix Modification – pH Adjustment

The effect of pH was investigated as means to enhance the extraction of the analytes. The 10ml solutions were analyzed triplicate in both acidic and basic conditions; pH 4.6 and pH 10.0 by addition of buffers. The acidic buffer was acetic acid-sodium acetate and the basic buffer was sodium bicarbonate-sodium carbonate; both buffers were prepared according to Jeffery *et al.* (1989).

The pH adjusted solutions were then extracted at 40°C for 30 min and desorped in the injector for 15 min at 270°C. Triplicate of solutions without pH adjustment (pH 6.8) were determined under the same condition. Carry over study was done after every extraction run to determine the completeness of desorption.

Preparation of buffers (Jeffery et al., 1989)

Buffer for ***pH 4.6***
= 0.10 M Acetic acid (CH_3CO_2H) + 0.10 M Sodium acetate (CH_3CO_2Na)

Buffer for ***pH 10.0***
= 0.01 M Sodium bicarbonate ($NaHCO_3$) + 0.025 M Sodium carbonate (Na_2CO_3)

The analytes were then spiked into the pH adjusted solution for analysis.

Figure 3.9: Preparation of buffers

Matrix Modification – Ionic Strength Correction

The extraction efficiency of the ionic strength effect was determined by analyzing samples which contained different amount of NaCl in the range from 5 to 25% (w/v). The triplicates of ionic strength adjusted solutions were extracted at 40°C for 30 min and desorped in the injector for 15 min at 270°C. Carry over study was done after every extraction run to determine the completeness of desorption.

Calculation for the preparation of ionic solutions (Skoog et al., 1994)

$$\text{weight / volume percent (w/v)} = \frac{\text{mass of solute, g}}{\text{volume of solvent, ml}} \times 100\%$$

For example: To prepare a 15% (w/v) NaCl solution in 100ml of distilled water

$$15\%\ (w/v) = \frac{x \text{ gram of solute}}{100 \text{ ml of solvent}} \times 100\%$$

$$x = 15 \text{ gram of salt}$$

→ *Dissolve 15 gram of NaCl in 100 ml distilled water to prepare a 100 ml of 15% (w/v) NaCl solution.*

Figure 3.10: Calculation for the preparation of ionic solutions.

3.4.3 Gas chromatograph conditions

Figure 3.11: Shimadzu® GC-17A gas chromatograph fitted with electron capture detector. Next to the GC is Shimadzu CBM-102 communications bus module.

Chromatographic analyses were performed using a Shimadzu GC–17A gas chromatograph fitted with a Ni^{36}-source electron capture detector at 300°C (Figure 3.11). A split/splitless injector in the splitless mode was used and it was held isothermally at 270 ºC. Thermogreen LB-2 septum Shimadzu plugs and a SPME GC column inlet for Shimadzu®17A with splitless injector (length 95 mm x O.D. 5 mm x I.D. 0.75 mm) from Supleco were used.

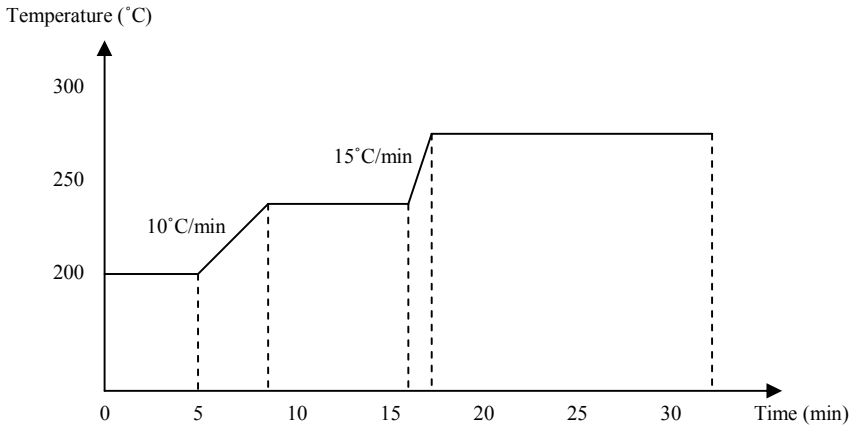


Figure 3.12: Temperature program of the gas chromatograph condition.

Analytes were separated using a SGE BPX5 0.25mm ID x 30 m length with a film thickness of 0.25μm capillary column. As shown in Figure 3.12, the column oven was programmed at 200°C hold for 5 min, 10°C/min to 240°C hold for 7 min, 15°C/min to 270°C hold for 15 min. Total run time was 33 min. 99.999% purity nitrogen was used as the carrier gas at 11.7 cm/s velocity.

Analysis of the chromatograms was performed utilizing Shimadzu® GCsolution Chromatography Data System Version 2.2. The gas chromatograph was connected to a PC through Shimadzu® CBM-102 communications bus module integrator.

3.4.4 Methods validations

One important step to ensure the quality and acceptability of the analytical results released by a laboratory is the use of analytical methods with performance capabilities consistent with the application requirements. The objective demonstration that the particular requirements for a specific intended use are fulfilled is achieved by means of method

validation. This demonstration is usually carried out through a series of laboratory experiments in which different performance characteristics of the method (e.g. accuracy, precision, and linearity) are assessed. Guiding principles and general requirements for validation of analytical methods have been proposed by different national and international organizations and regulatory authorities. The differences in the aims of the analysis between quantitative and qualitative method considerably reduce the list of validation parameters for qualitative methods. The extent of the validation depends on the aim of the analytical method, and the first step is to decide which performance parameters must be studied and then design the validation procedure accordingly (Jimenez *et al*, 2002).

Table 3.1: Validation parameters for qualitative methods according to the requirements and recommendations of different national and international organization (Jimenez *et al*, 2002).

Validation parameter	**ENAC**	**ICH**	**UN**
Accuracy	-	-	X
Precision/repeatability	-	-	X
Specificity/selectivity	X	X	X
Range	-	-	-
Linearity	-	-	-
Limit of detection (LOD)	X	X	X
Limit of quantitation (LOQ)	-	-	X
Ruggedness	-	-	X
Recovery	-	-	-

ENAC – Entidad Nacional de Acreditacion (Spanish Accreditation Body); ICH – International Conference on Harmonization; UN – United Nations Drug Control Programme.

According to Table 3.1, the essential parameters needed to evaluate the overall performance of a qualitative analytical method are selectivity and LOD. Additionally, extraction recovery, precision and linearity have been included in the strategy for validation of qualitative analytical methods.

3.4.4.1 Optimized SPME conditions

The SPME method was optimized and validated at the following operational conditions: 10 ml of sample are extracted by immersion of a 100 μm PDMS fiber for 30 min; sample agitation was employed at 600 rpm and temperature control at 40°C; neither pH adjustment nor ionic strength correction were applied. The PDMS fiber was then inserted in the injector at 270°C for 12 min. The total GC run time was 33 min with a two-step temperature program.

3.4.4.2 Precision/Repeatability

Precision is a measure of the closeness with which multiple analyses of a given sample agree with each other. Assess precision by replicate analyses, by repeated analyses of a stable standard, or by analysis of known additions to samples. Precision is specified by the standard deviation (SD) of the results (Clesceri *et al.*, 1989). Other than standard deviation, precision may also be expressed in different term as relative standard deviation (RSD) (Csuros, 1994).

Since repeatability RSD of ± 15% was used as the acceptance criterion for samples at high concentrations of the analytes. However, due to the special characteristics, the complexity and the objectives of the qualitative methods evaluated, a wider acceptance criterion was proposed for the low concentration samples, and RSD values for ± 20 – 25 % were accepted (Jiménez *et al.*, 2002).

3.4.4.3 Accuracy/Recovery

Accuracy is the degree of agreement of a measured value with the true or expected value of the quantity of concern. Accuracy is measured and expressed as % recovery (Csuros, 1994). Because SPME is an non-exhaustive extraction procedure, relative recovery, determined as the peak area ratio for the real sample and for Milipore filtered distilled water spiked with analytes at the same level (instead of absolute recovery as used in exhaustive extraction procedures) was employed.

The recoveries for all studied analytes except dichlorvos and azinphos ethyl, were determined at high (20 ppb), medium (5 ppb) and low (1 ppb) concentrations, using triplicates for each evaluated concentration under optimized conditions as described in section 3.4.4.1. On the other hand, the recoveries for dichlorvos and azinphos ethyl were determined at high (100 ppb), medium (50 ppb) and low (20 ppb) concentrations using triplicates for each evaluated concentration under optimized conditions as described in section 3.4.4.1.

3.4.4.4 Limits of detection (LOD)

The detection limit of a method is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically, three times the noise level. The detection limit needs to be determined only for impurity methods in which chromatographic peaks near the detection limit will be observed. An example of a detection limit criteria is that, at the 0.05% level, an impurity will have S/N ratio of 3:1 (Green, 1996).

The LOD were determined by serial dilution analysis as described below and not by calculations based on S/N ratios. Solutions of the standard compounds were prepared by dissolving the working solution in Milipore filtered distilled water and diluted to 2, 1, 0.5, 0.1, 0.05, 0.01 ppb for all analytes except dichlorvos and azinphos ethyl; and 10, 5, 2, 1.0, 0.5 ppb for dichlorvos and azinphos ethyl. The LOD values for each pesticide were achieved under optimized conditions as described in section 3.4.4.1.

3.4.4.5 Linearity

Linearity is defined as the ability of the method, within a given range, to obtain an acceptable linear correlation between the results and the concentration of analyte in samples. For assay methods, this study is generally performed by preparing standard solutions at five concentration levels, from 50 to 150% of the target analyte concentration. Five levels are required to allow detection of curvature in the plotted data. The standards are evaluated using the chromatographic conditions determined during the specificity studies. Standards should be prepared and analyzed a minimum of three times (Green, 1996).

Linearity of the analytes (except dichlorvos and azinphos ethyl) were determined at 0.1, 0.5, 1, 5, 10, 15, 20 and 15, 30, 50, 80, 120, 150 ppb for dichlorvos and azinphos ethyl respectively, under optimized conditions as described in section 3.4.4.1.

3.4.5 Sample analysis

Standard addition method was initially applied for the sample analysis. In the presence of matrix interference, the application of standard addition technique is useful. In this technique, the accurate concentration of the analyte is obtained without the elimination of

the interfering substance. Aliquot of standards are added to portions of the sample allowing the interfering substance in the sample also affect the standard (Csuros, 1994).

The main consideration of applying standard addition method was the appearance of salinity and the pH in the water samples which are part of the extraction parameters and might affect SPME extraction efficiency.

In SPME method, volume of sample extracted is very important. The total SPME extraction volume of sample in this study is 10ml. In standard addition method, 9 ml of water sample was taken then a series of increasing volumes of working standard were added to the 10ml volumetric flask. Finally, each flask was made up to the mark with Milipore filtered distilled water and mixed well.

Concentrations of analytes added to both the sample and Milipore filtered distilled water were 1, 5, 10, 15, 20 ppb for all analytes except dichlorvos and azinphos ethyl; and 70, 80, 90, 100, 110 ppb for both dichlorvos and azinphos ethyl. The extractions were done under optimized conditions as described in section 3.4.4.1.

Although standard addition method is suggested to be a better quantification technique as the accurate concentration of the analyte is obtained without the elimination of the interfering substance, external standard calibration method was also done with the consideration of its simplicity and the samples involved are simple matrices. If the results of both quantification techniques are satisfactory, the simpler quantification would be chosen for the quantification of samples.

A series of working standards of the analytes were prepared by appropriate dilution from 1 ppm of standard solutions (for all analytes except dichlorvos and azinphos ethyl); and 10 ppm for dichlorvos and azinphos ethyl, to yield concentrations of 1, 5, 10, 15, 20 ppb (for all analytes except dichlorvos and azinphos ethyl); and 70, 80, 90, 100, 110 ppb for both dichlorvos and azinphos ethyl in Milipore filtered distilled water. These standards were subjected to SPME procedure and injected into the GC-ECD under optimized conditions as described in section 3.4.4.1. Determination of each analyte concentration was repeated 7 times.

CHAPTER 4 – RESULTS AND DISCUSSION

4.1 Sampling parameters

Physical parameters of the water samples during every sampling expedition were collected and compiled as follows:

Table 4.1: *In-situ* parameters during 1st sampling on 27th May 2005

Sampling point	1st sampling (27 May 2005)						
	Latitude (N)	Longitude (E)	Temperature (˚C)	pH	DO[a] (mg/l)	Salinity (‰)	Time
1	04˚ 16.84’	100˚ 40.25’	30.7	7.11	2.9	29	10.34 am
2	04˚ 16.35’	100˚ 40.18’	30.7	7.19	2.7	28	10.40 am
3	04˚ 15.30’	100˚ 40.15’	30.4	7.31	2.8	28	10.44 am
4	04˚ 15.23’	100˚ 40.19’	30.9	7.20	3.8	27	10.49 am
5	04˚ 15.20’	100˚ 40.33’	30.7	7.18	2.3	26	10.59 am
6	04˚ 16.00’	100˚ 41.26’	30.3	7.03	0.9	25	11.12 am
7	04˚ 15.47’	100˚ 41.86’	30.5	6.82	1.2	25	11.50 am
8	04˚ 16.42’	100˚ 40.46’	31.7	7.36	4.1	26	12.05 pm
9	04˚ 17.40’	100˚ 39.32’	31.8	7.59	5.6	28	12.25 pm
10	04˚ 16.17’	100˚ 39.30’	31.5	7.59	6.0	28	12.15 pm

[a]: DO = dissolved oxygen

Table 4.2: *In-situ* parameters during 2nd sampling on 12th October 2005

Sampling point	2nd sampling (12 October 2005)						
	Latitude (N)	Longitude (E)	Temperature (˚C)	pH	DO[a] (mg/l)	Salinity (‰)	Time
1	04˚ 16.24’	100˚ 40.66’	30.6	5.87	4.4	28	9.04 am
2	04˚ 16.08’	100˚ 40.55’	30.4	6.18	2.4	29	9.17 am
3	04˚ 15.78’	100˚ 40.41’	30.3	6.70	3.6	29	9.27 am
4	04˚ 15.65’	100˚ 40.53’	30.1	6.84	3.7	29	9.34 am
5	04˚ 15.54’	100˚ 40.90’	30.3	6.88	3.4	29	9.47 am
6	04˚ 15.34’	100˚ 41.69’	30.7	6.79	1.5	28	10.00 am
7	04˚ 14.98’	100˚ 41.28’	30.2	6.77	2.7	28	10.13 am
8	04˚ 15.92’	100˚ 40.11’	30.3	6.94	4.9	29	10.33 am
9	04˚ 16.53’	100˚ 39.76’	30.4	7.12	5.2	29	10.47 am
10	04˚ 17.14’	100˚ 39.88’	30.5	7.13	4.8	29.5	10.57 am

[a]: DO = dissolved oxygen

Table 4.3: *In-situ* parameters during 3rd sampling on 08th December 2005

Sampling point	3rd sampling (08 December 2005)						
	Latitude (N)	Longitude (E)	Temperature (˚C)	pH	DO[a] (mg/l)	Salinity (‰)	Time
1	04˚ 16.24’	100˚ 40.63’	28.7	5.98	2.2	23	9.11am
2	04˚ 16.06’	100˚ 40.55’	29.6	5.95	2.1	24	9.20 am
3	04˚ 15.66’	100˚ 40.49’	29.5	5.96	2.2	22	9.28 am
4	04˚ 15.64’	100˚ 40.55’	28.9	6.25	2.0	17	9.31 am
5	04˚ 15.53’	100˚ 40.92’	28.8	6.27	1.6	17	9.42 am
6	04˚ 15.30’	100˚ 41.69’	28.9	6.14	0.3	14	9.56 am
7	04˚ 14.97’	100˚ 41.28’	28.4	6.22	1.2	24	10.11 am
8	04˚ 15.88’	100˚ 40.10’	29.3	6.21	2.2	28	10.20 am
9	04˚ 16.90’	100˚ 39.83’	29.7	6.21	3.1	27	10.41 am
10	04˚ 17.19’	100˚ 39.93’	29.4	6.33	3.3	27	11.00 am

[a]: DO = dissolved oxygen

Water sample collection varies depending on the collection source. When a water sample collected from a river is analyzed, it is necessary to keep in mind that the concentrations of the elements under study will vary according to the depth, the stream speed, the distance from the bank and the width of the river. This is why the site and the position of the sampling should be registered exactly to obtain representative samples during the sampling period (Meyers, 2000).

Table 4.4 (a), (b), and (c) presented the precision of every sampling point through mathematical calculations from the GPS readings obtained throughout the 3 sampling expeditions. Latitude variance of the sampling locations ranged between 0.16’ and 0.58’ with relative standard deviation of 1.00% - 3.43%. On the other hand, variance of the longitude for the sampling locations ranged between 0.18’ and 0.35’ with relative standard deviation of 0.44 % - 0.88 %.

Table 4.4 (a): Latitude (N) ± standard deviation (SD) of sampling points

Sampling point	Latitude (N)			Average latitude (N) ± SD
	1st sampling	2nd sampling	3rd sampling	
1	04˚ 16.84	04˚ 16.24	04˚ 16.24	04˚ 16.44’ ± 0.35’
2	04˚ 16.35	04˚ 16.08	04˚ 16.06	04˚ 16.16’ ± 0.16’
3	04˚ 15.30	04˚ 15.78	04˚ 15.66	04˚ 15.58’ ± 0.25’
4	04˚ 15.23	04˚ 15.65	04˚ 15.64	04˚ 15.51’ ± 0.24’
5	04˚ 15.19	04˚ 15.54	04˚ 15.53	04˚ 15.42’ ± 0.20’
6	04˚ 15.96	04˚ 15.34	04˚ 15.30	04˚ 15.53’ ± 0.37’
7	04˚ 15.47	04˚ 14.98	04˚ 14.97	04˚ 15.14’ ± 0.29’
8	04˚ 16.42	04˚ 15.92	04˚ 15.88	04˚ 16.07’ ± 0.30’
9	04˚ 17.41	04˚ 16.53	04˚ 16.90	04˚ 16.95’ ± 0.44’
10	04˚ 16.17	04˚ 17.14	04˚ 17.19	04˚ 16.83’ ± 0.58’

Table 4.4 (b): Longitude (E) ± standard deviation (SD) of sampling points

Sampling point	Longitude (E)			Average longitude (E) ± SD
	1st sampling	2nd sampling	3rd sampling	
1	100˚ 40.25	100˚ 40.66	100˚ 40.63	100˚ 40.51’ ± 0.23’
2	100˚ 40.18	100˚ 40.55	100˚ 40.55	100˚ 40.42’ ± 0.21’
3	100˚ 40.15	100˚ 40.41	100˚ 40.49	100˚ 40.35’ ± 0.18’
4	100˚ 40.19	100˚ 40.53	100˚ 40.55	100˚ 40.42’ ± 0.20’
5	100˚ 40.33	100˚ 40.90	100˚ 40.92	100˚ 40.72’ ± 0.34’
6	100˚ 41.26	100˚ 41.69	100˚ 41.69	100˚ 41.55’ ± 0.25’
7	100˚ 41.86	100˚ 41.28	100˚ 41.28	100˚ 41.47’ ± 0.34’
8	100˚ 40.46	100˚ 40.11	100˚ 40.10	100˚ 40.23’ ± 0.20’
9	100˚ 39.32	100˚ 39.75	100˚ 39.83	100˚ 39.64’ ± 0.28’
10	100˚ 39.30	100˚ 39.88	100˚ 39.93	100˚ 39.70’ ± 0.35’

Table 4.4 (c): Summary of the sampling locations with standard deviations (SD) and relative standard deviations (RSD)

Sampling point	Latitude (N)		Longitude (E)	
	Latitude (N) ± SD	RSD (%)	Longitude (E) ± SD	RSD (%)
1	04˚ 16.44’ ± 0.35’	2.10	100˚ 40.51’ ± 0.23’	0.56
2	04˚ 16.16’ ± 0.16’	1.00	100˚ 40.42’ ± 0.21’	0.52
3	04˚ 15.58’ ± 0.25’	1.62	100˚ 40.35’ ± 0.18’	0.44
4	04˚ 15.51’ ± 0.24’	1.54	100˚ 40.42’ ± 0.20’	0.50
5	04˚ 15.42’ ± 0.20’	1.30	100˚ 40.72’ ± 0.34’	0.82
6	04˚ 15.53’ ± 0.37’	2.38	100˚ 41.55’ ± 0.25’	0.60
7	04˚ 15.14’ ± 0.29’	1.91	100˚ 41.47’ ± 0.34’	0.81

8	04° 16.07' ± 0.30'	1.87	100° 40.23' ± 0.20'	0.51
9	04° 16.95' ± 0.44'	2.59	100° 39.64' ± 0.28'	0.70
10	04° 16.83' ± 0.58'	3.43	100° 39.70' ± 0.35'	0.88

4.2 Solid-phase microextraction (SPME)

4.2.1 Fiber selection

The organochlorines under investigation fall into a non-polar class with relatively high octanol-water coefficients (log P_{ow}), and very low solubility in water. Hence, these analytes would be expected to partition more readily into a more non-polar fiber coating rather than a polar one. The polydimethylsiloxane polymeric coating was selected for the extraction of these analytes from the aqueous medium (Magdic and Pawliszyn, 1996).

The PDMS fiber is the most common nonpolar phase which is similar to OV®-1 and SE-30 type GC phases (Wercinski, 1999). Advantages of these phases for SPME applications are similar to the advantages in their use as GC stationary phases. They are very rugged liquid coatings which are able to withstand high injector temperatures, up to about 300°C. PDMS is a non-polar phase and it extracts nonpolar analytes very well. However, it also can be applied successfully to more polar compounds, particularly after optimizing extraction conditions (Pawliszyn, 1997).

According to Pawliszyn (1997) fiber selection guidelines, 100µm PDMS fiber is suitable to extract pesticides which contain phosphorus or chlorinated. The usability of PDMS fiber on extracting organochlorine and organophosphorus pesticides has been proven by several researchers (Boyd-Boland et al., 1996; Magdic *et al.*, 1996; Beltran *et al.,* 1998; Lambropoulou and Albanis, 2001; Lambropoulou *et al.*, 2002).

4.2.2 Optimization of SPME parameters

4.2.2.1 The optimum parameter

The optimum SPME method for the GC-ECD analysis of selected analytes utilizing PDMS fiber was found to be 30 min of extraction at 40˚C under continuous stirring condition; 12 min of desorption at 270˚C. None of matrix modifications were applied in this study.

SPME is an equilibrium process that involves the partitioning of analytes from a liquid or gaseous sample into the polymeric phase according to their partition coefficients, *K*. The SPME process can be described by the following formula (Magdic and Pawliszyn, 1996):

$$n_s = \frac{KV_sV_{aq}C^0_{aq}}{KV_s + V_{aq}} \qquad (1)$$

where n_s is the amount extracted by the fiber coating, V_{aq} and V_s are the volumes of the aqueous phase and stationary phase, respectively, and C^0_{aq} is the initial concentration of the analytes in the aqueous phase. Equation 1 indicates that the amount of analytes extracted is dependent on both the volume of the stationary phase and the partition coefficient, *K*. Likewise the sensitivity and the linear range of the method are also dependent upon these parameters. Therefore, the selection of an appropriate stationary phase is extremely important.

Since SPME is a process dependent on equilibrium rather than total extraction, the amount of analyte extracted at a given time is dependent on the mass transfer of an analyte through the aqueous phase (Magdic *et al.*, 1996).

All experiments were performed under agitation in order to optimize the transfer of analytes from the aqueous sample into the fiber coating.

4.2.2.2 Desorption step and carryover study

Extraction time and temperature are the primary factors governing the fiber-SPME-GC desorption (Krutz *et al.*, 2003). Optimization of desorption time was repeated whenever the extraction conditions were changed. Longer desorption might be needed when the extraction efficiencies were improved. It is due to the extraction of analytes was not done at equilibrium, increased extraction

Desorption Time

In this study the optimization of desorption time was done by leaving the SPME fiber in the injector for lengths of time ranging from 2 to 16 min at 2 min intervals after the extraction of mixed standard solutions. Desorption was done at 270˚C. With the consideration of the presence of carryover, a blank (the fiber was placed in the injector without prior exposure to the sample) following the initial desorption was routinely applied between the extractions to determine the presence of carry over.

Efficiency of desorption period was determined by observing the presence of the analytes peak in the chromatogram after desorption of blank was performed. When the peak of the analytes was observed indicating the presence of carryover and longer desorption time would be needed for the next investigation. The experiment was done in triplicate for each of the desorption time.

Figure 4.1 showed carry over profile of the peak area observed after the desorption of each blank analysis. Desorption time of 2 to 6 minutes was insufficient as trace of analytes were still observed in the blank run. Carry over of dichlorvos and azinphos ethyl were no more observed after desorption time of 10 minutes whilst carry over of the rest of analytes were no more observed after desorption of 12 minutes.

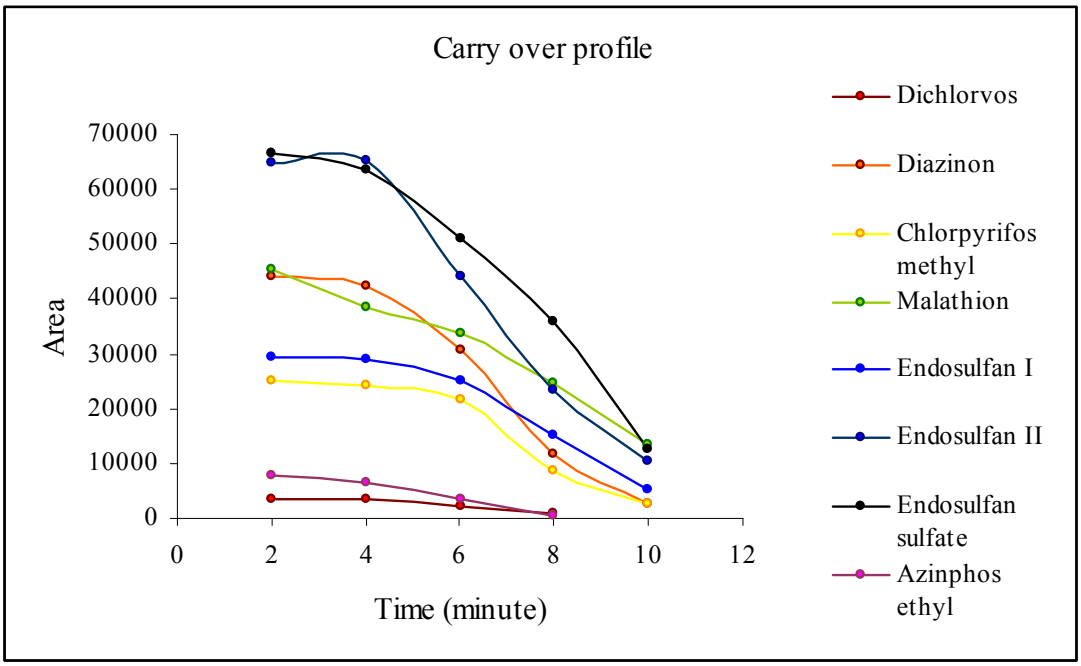


Figure 4.1: Carry over profile of the mixed standard solution from Milipore filtered distilled water with 100 µm PDMS fiber.

For the optimum extraction condition of 30 min extraction at 40˚C without matrix modifications, a desorption period of 12 min was found to be enough to desorb the analytes from the PDMS fiber. No significant carryover of any pesticide was observed after 12 min of desorption.

According to Voler *et al.* (1997), the carryover problem becomes significant when low volatility compounds are analyzed. They had studied the carryover effect by running a blank after an extraction of 2 ppb of the organophosphorus pesticides and found the

carryovers obtained were lower than 0.1% with the exception of diazinon (3.0%). The problem could however be reduced by enlarging the desorption time or by running a blank after the calibration or contaminated samples. Some carry over problems difficult to eliminate even after the running of several blanks have been attributed to chemisorption process (Voler *et al.*, 1997). Wercinski (1999) also suggested by desorbing the fiber for as long as 15 – 20 minutes, the significant sample carryover can be reduced to an acceptable level (> 0.5%).

Desorption Temperature

Aguilar *et. al.*(1998) stated that the desorption of an analyte from an SPME fiber depends on its boiling point and also on the temperature of the injection port; those compounds with higher boiling points are successfully desorbed at higher temperature.

However, no investigation of desorption temperature was carried out in this study. The selection of desorption temperature was based on literature studied. According to experiment done by Beltran *et al.* (1998), 270˚C was found to be the optimum desorption temperature of 100 μm PDMS fiber for organophosphorus pesticides. Page and Lacroix (1997) employed injector temperature programming from 60˚C to 250˚C at 60˚C/min with a 23-min hold for the fiber desorption of semi-volatile organochlorine contaminants. On the other hand, Boussahel *et al.* (2002) used injector temperature of 220˚C to desorb organochlorinate pesticides. Boyd-Boland *et al.* (1996) set the injector temperature at 250˚C for the determination of 60 pesticides which included organophosphorus and organochlorine pesticides.

The desorption temperature was set at 270˚C with the consideration of the GC temperature program. Separation of analyte mixture by GC was optimized with 2-step temperature programming where the final temperature was 270˚C. According to the GC manufacturer's instruction manual (Shimadzu, 1995), the injector temperature should set higher than the column temperature to prevent contamination of injector. Wercinski (1999) also stated that injector temperature is normally 10 – 20˚C below the temperature limit of the fiber and/or the GC column which is usually 200˚C to 280˚C. As a result the minimum injector temperature was set at 270˚C due to the temperature program.

4.2.2.3 Extraction step

Extraction Temperature

The effect of temperature was studied by sampling the mixed standard solutions under different temperature conditions ranged between 30˚C and 70˚C. No matrix modification was done. The analytes were then thermally desorped into the injection port of a gas chromatograph at 270°C for 12 min.

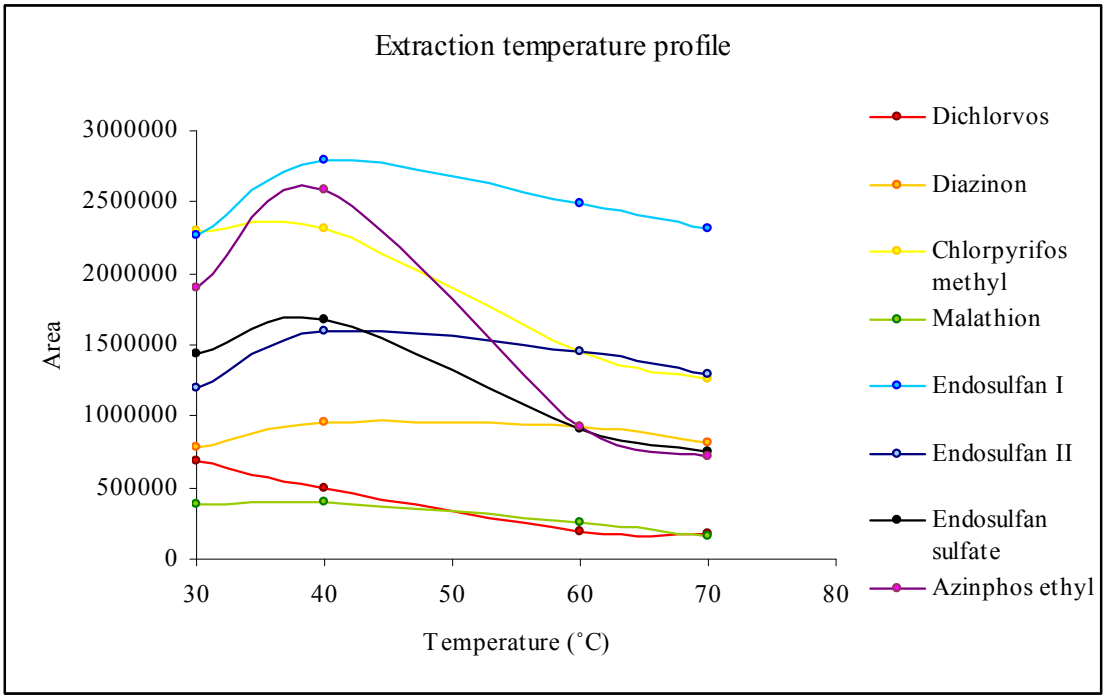


Figure 4.2: Temperature effect in the extraction of the mixed standard solutions from Milipore filtered distilled water with 100 µm PDMS fiber.

Figure 4.2 shows the GC areas obtained for the eight analytes at the different temperatures. Triplicate analyses were made at each extraction temperature. An increase in extraction efficiency of the analytes was observed in most of the analytes when the temperature increases until 40°C. This may be attributed to the increase in extraction temperature decreases the partition coefficient between analytes and water (Dong *et al.*, 2005). On the other hand, Dong *et al.* (2005) also studied that a decrease in sensitivity was also observed for the studied analytes when the extraction temperature exceeded 60°C. This is because adsorption is an exothermic process and therefore, disfavored at high temperature.

Voler *et al.* (1997) stated the diffusion of the analytes in the aqueous phase increases as temperature rises. Thus, the extraction limited basically by mass transfer, is more efficient at higher temperatures. However, the absorption is an exothermic process and increasing

temperature has a negative effect in such processes. Zhang and Pawliszyn (1995), reported better conditions can be obtained by heating the sample and internally cooling the fiber to improve the analyte diffusion and to favor the exothermic process. However, such a system is difficult to realize and real benefits are poor.

Sauret-Szczepanski *et al.* (2006), also concluded that the decrease of extraction efficiency above 50˚C were probably the result of competition between the kinetics of adsorption and desorption from the fiber. Indeed, the principle of SPME is based on the equilibrium of the analytes between the solid and liquid phases. This equilibrium is ruled out by the kinetics of adsorption and desorption from the fiber. Because these kinetics are temperature dependent, the variations of the working temperature modify the solid-liquid equilibrium and consequently the extraction efficiency. According to their results, it seems that, up to 50˚C the increasing temperature is favorable to the kinetics of adsorption while above this value, the kinetic competition becomes favorable to the desorption mechanism.

From the study, dichlorvos was observed where the extraction efficiency slowly decreased start from the beginning of the temperature – absorption profile. This may be explained by the high solubility of the analyte in water (~ 1000 mg/l at room temperature; Verschueren, 2001). Elevated temperature might had had enhanced the analyte solubility in water and caused poor extraction efficiency.

Air bubbles were observed at temperature above 50˚C. According to Aguilar *et al.* (1998), the appearance of air bubbles can significantly affect the precision if they are adsorped at the stationary phase so they should be avoided and removed before the fiber is exposed to the sample.

As a result, the temperature chosen in this study was 40˚C since under these conditions the peak area for most of the pesticides had a maximum value.

Extraction time

The extraction time was studied by sampling of the analytes from the mixed standard solutions under different period of time ranged from 5 min to equilibrium. The analytes were then thermally desorped into the injection port of a gas chromatography at 270°C for 12 min.

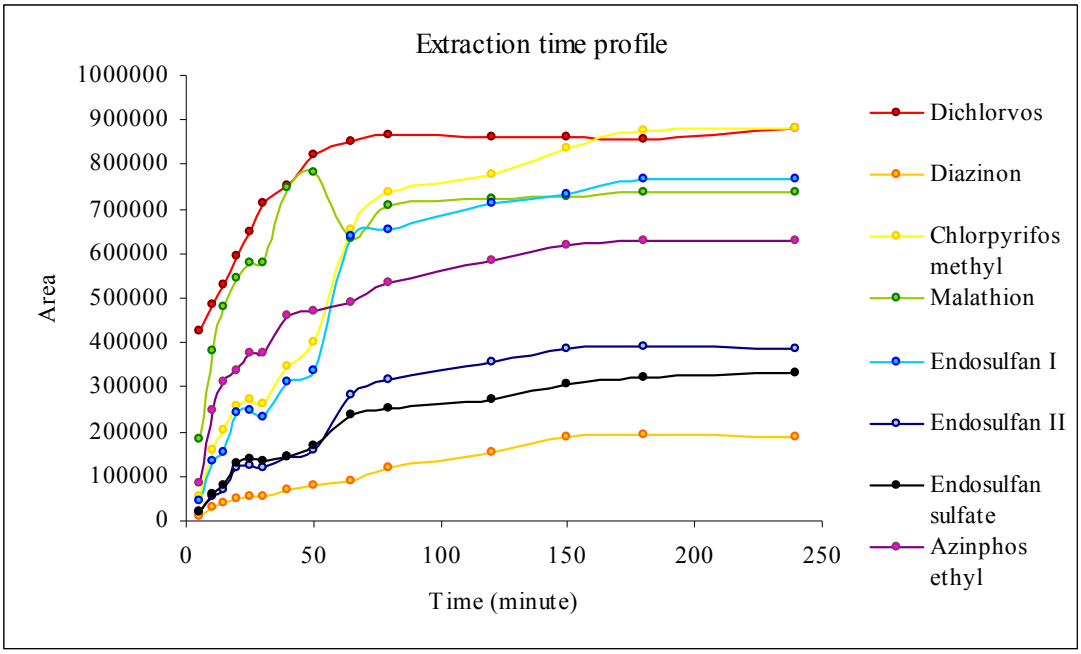


Figure 4.3: The effect of extraction time for the mixed standard solution from Milipore filtered distilled water with 100 µm PDMS fiber.

The extraction time profile obtained using the 100 µm PDMS fiber is shown in Figure 4.3. The equilibrium condition for the absorption of most analytes is almost reached after 150 minute except dichlorvos and malathion 80 minute; and chlorphyrifos methyl, 180 minute. Analytes with higher log P_{ow} were the more extensively absorbed at equilibrium due to their higher affinity to the fiber coating, so will have long equilibration times because more

analytes must travel into the fiber (Wercinski, 1999). On the other hand, dichlorvos and malathion have lower log P_{ow} reached equilibrium faster.

Wercinski (1999) reported because maximum productivity is required in most laboratories, GC run times should be as short as possible; therefore, the SPME sampling time should be no longer than the total GC cycle time, minus the desorption time. Good precision can be achieved without attaining equilibrium if the equilibrium timing is precisely controlled. Pawliszyn (1997) also stated that when using a shorter extraction time compared to equilibration time, care must be taken to control the exposure time and the longest possible extraction time should be applied. Constant convection and temperature in the system needs to be ensured to obtain reproducible data. This condition requires good temperature control and constant agitation.

A count up/down digital timer was employed to ensure the absorption period is accurate to within ± 1 second.

As a result, with the consideration of the lab work efficiency the extraction period of 30 minute was selected since it was approximately equivalent to the time required to run the GC chromatogram. Also, according to Valor *et al.* (1997), the use of equilibrium time in the absorption phase can be unnecessary if LOD and RSD values obtained are acceptable.

Matrix Modification – pH Adjustment

The effect of pH was investigated as a means to enhance the extraction of the analytes. The 10 ml solutions were analyzed triplicate at both acidic and basic conditions; pH 4.6 and pH 10.0 respectively, by addition of buffers. The acidic buffer was acetic acid-sodium acetate and the basic buffer was sodium bicarbonate-sodium carbonate. The pH adjusted solutions were then extracted at 40°C for 30 min and desorped in the injector for 12 min at 270°C. Triplicate of solutions without pH adjustment (pH 6.8) were determined under the same condition.

Matrix pH can be adjusted to optimize the SPME of acidic and basic pesticides. Extraction efficiency for acidic pesticides increases as pH decreases. At low pH, the acid-base equilibria of acidic pesticides are shifted toward the neutral form and analyte partitioning into the stationary phase is enhanced. Conversely, basic pesticides shift towards the ionized form as pH decreases and extraction efficiency decreases (Krutz *et al.*, 2003).

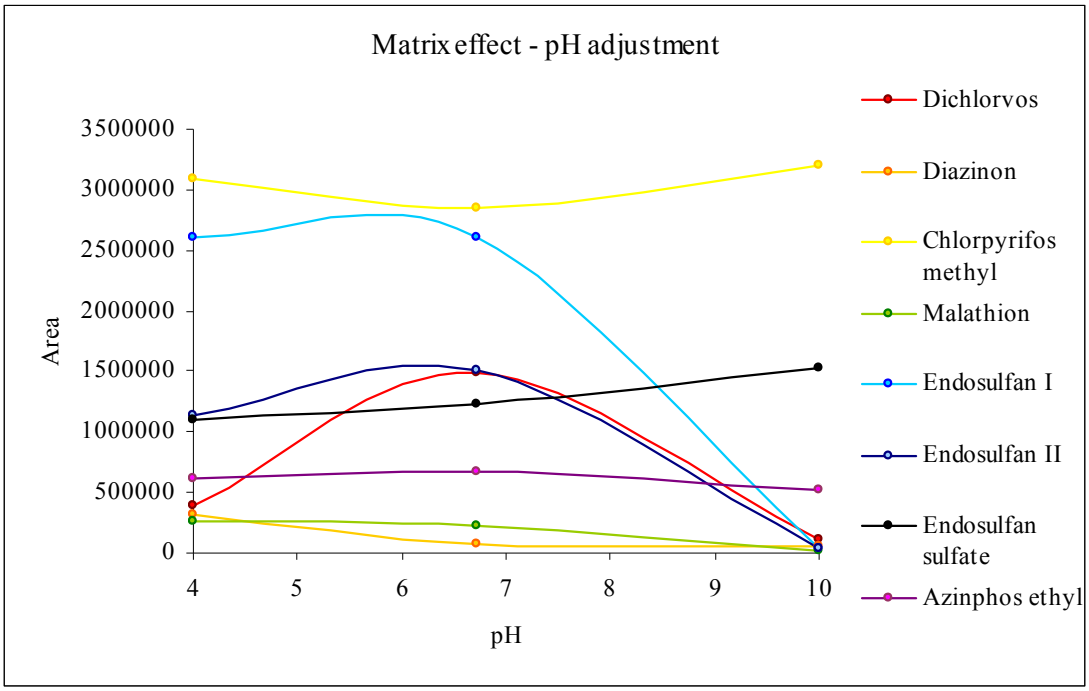


Figure 4.4: The effect of pH on the extraction of the mixed standard solutions with 100 µm PDMS fiber under different pH conditions.

Figure 4.4 shows the effects of pH in extraction efficiency. When the pH was lowered to 4.6, the response obtained for some of the analytes such as dichlorvos, endosulfan I and endosulfan II decreased. On the other hand, diazinon and chlorpyrifos methyl, showed a slightly increase in response. For malathion, endosulfan sulfate and azinphos ethyl, the acidic conditions did not affect the GC response of the analytes. The analytes did not showed obvious response in basic conditions except for dichlorvos, endosulfan I and endosulfan II which the response decreased at basic conditions.

Further analyses were carried out without adjusting the pH since most analytes have an acceptable response at neutral (without pH adjustment) condition, although some compounds response better under acidic or basic conditions.

Matrix modification – Ionic Strength Adjustment

The extraction efficiency of the ionic strength effect was determined by analyzing samples which contained different amount of NaCl in the range from 0 to 20% (w/v). The triplicates of ionic strength adjusted solutions were extracted at 40°C for 30 min and desorped in the injector for 12 min at 270°C.

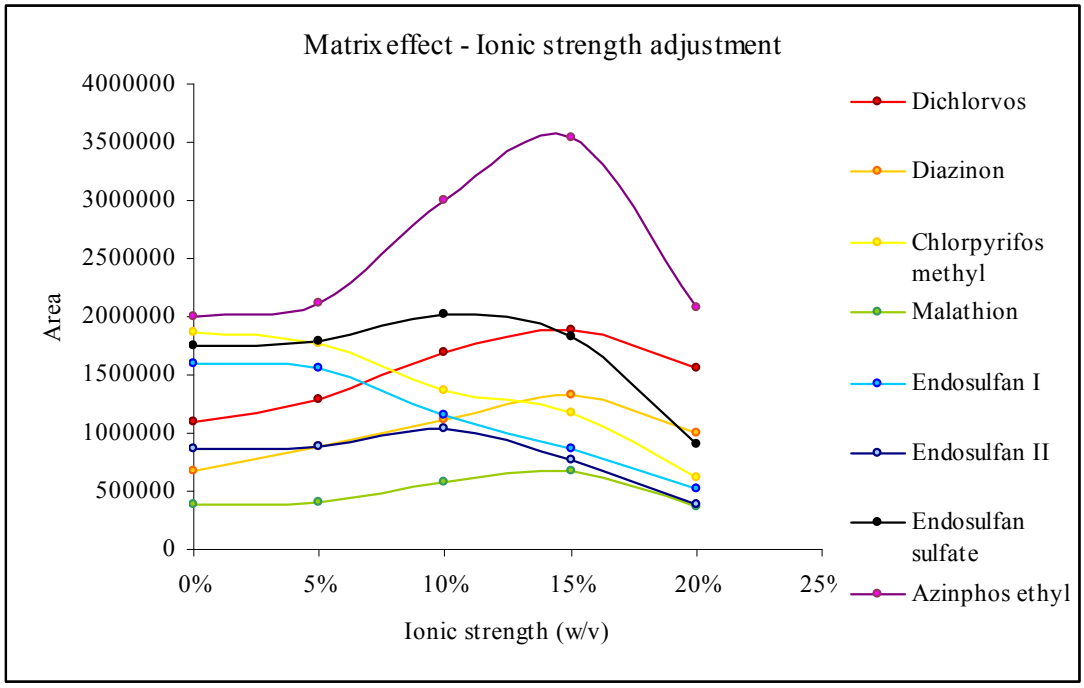


Figure 4.5: The effect of ionic strength to the extraction of mixed standard solution with 100 µm PDMS fiber under ionic strength (w/v) variations.

The result on the effect of NaCl concentration added to the solutions as the salting out agent for the 100 µm PDMS fiber is shown in Figure 4.5. The effect of ionic strength adjustment on the analytes has a relationship with their solubilities in the aqueous phase (Santos and Galceran, 1996). The greater the solubility of analytes in water the greater the influence on adsorption will be by adding salt. The compounds with higher water solubility (dichlorvos, diazinon, malathion and azinphos ethyl) showed an increase in extraction yield with the addition of increasing NaCl concentration until 15% (w/v). However, no effect or even a decrease in extraction yield was observed for compounds of

low water solubility (chlorpyrifos methyl, endosulfan I, endosulfan II and endosulfan sulfate) after 5% (w/v) of NaCl addition.

In SPME procedure the salting-out effect can be employed to modify the matrix by adding salt, e.g. Na_2SO_4 and NaCl to increase the ionic strength of the water so as to decrease the solubility of analytes and release more analytes from the samples and hence enhance the adsorption of the fiber (Magdic *et al.*, 1996; Lambropoulou and Albanis, 2001; Lambropoulou *et al.,* 2002).

However, Pawliszyn (1997) stated that salting can increase or decrease the amount extracted, depending on the compound and salt concentration, and the effect of salting on SPME has been determined to date only by experiment but has not been examined theoretically. In general, the salting effect increases proportionally with the polarity of the compound. Saturation with salt can be used not only to lower the detection limits of determination, but also to normalize random salt concentration in natural matrixes. Note that salting can lower pH at high salt concentration level, since proton activity is increased with increased solution ionic strength.

As a result, no ionic strength adjustment was applied in this study since half of the analytes showed decrease extraction efficiency when ionic strength was increased even though the other analytes showed increased extraction yields.

4.2.3 Method validation

4.2.3.1 Result of recovery test

Table 4.5: Recoveries of analytes under investigation

Analytes	n	Recoveries (mean ± SD), %					
		Low	RSD%	Medium	RSD%	High	RSD%
		1ppb		**5ppb**		**20ppb**	
Diazinon	3	107.25 ± 15.26	14.23	95.72 ± 8.49	8.87	103.17 ± 8.34	8.09
Chlorpyrifos methyl	3	109.60 ± 4.03	3.67	102.41 ± 16.54	16.15	97.89 ± 5.14	5.25
Malathion	3	106.96 ± 16.84	15.74	103.82 ± 7.44	7.17	100.25 ± 5.94	5.93
Endosulfan I	3	105.89 ± 17.41	16.43	95.06 ± 5.77	6.07	101.12 ± 4.98	4.92
Endosulfan II	3	112.47 ± 13.07	11.62	100.57 ± 15.67	15.58	95.34 ± 5.48	5.75
Endosulfan sulfate	3	124.29 ± 25.85	20.80	108.33 ± 14.29	13.19	102.63 ± 8.84	8.61
		20ppb		**50ppb**		**100ppb**	
Dichlorvos	3	94.14 ± 11.89	12.63	90.64 ± 3.89	4.30	96.08 ± 4.26	4.44
Azinphos ethyl	3	107.12 ± 12.57	11.73	118.06 ± 16.05	13.59	114.84 ± 13.39	11.66

The relative recoveries (mean ± SD) obtained from SPME extraction were presented in Table 4.5. Extraction recoveries were sufficient, ranging between 90.64% and 124.29%. The RSD for the compound were acceptable (< 25% for low concentration samples; < 15% for high concentration samples). The results suggested that there was no relevant difference in extraction recovery at different concentration levels for the analytes under investigation.

4.2.3.2 Result of limits of detection (LOD) test

Table 4.6: Limits of detection (LOD) of analytes under investigation

Analytes	Limit of detection (LOD), ppb
Dichlorvos	5.00
Diazinon	0.01
Chlorpyrifos methyl	0.01
Malathion	0.50
Endosulfan I	0.01
Endosulfan II	0.01
Endosulfan sulfate	0.01
Azinphos ethyl	5.00

The LOD obtained for the targeted analytes were shown in Table 4.6. The results obtained in the validation indicated that the criteria for the evaluation of the validation parameters also have to be defined in accordance with the intended purposes. As mentioned before, in accordance with the recommendations the most relevant validation parameters in qualitative method are the selectivity and the LOD, so that low extraction recoveries were accepted when the detection method was reproducible and sufficiently sensitive.

4.2.3.3 Result of linearity test

Table 4.7 Linearity range of analytes under investigation

Analytes	n	Linearity range (ppb)	Regression coefficient (R^2)
Dichlorvos	7	15.00 – 150.00	0.9711
Diazinon	7	0.10 – 15.00	0.9888
Chlorpyrifos methyl	7	0.10 – 15.00	0.9920
Malathion	7	1.00 – 20.00	0.9824
Endosulfan I	7	0.10 – 20.00	0.9913
Endosulfan II	7	0.10 – 20.00	0.9915
Endosulfan sulfate	7	0.10 – 15.00	0.9917
Azinphos ethyl	7	15.00 – 150.00	0.9911

The linearity range of the investigated analytes was shown in Table 4.7. The line of best fit for the relationship between the average peak area and the concentration of analyte in the sample was determined by linear regression. The procedure revealed linear behavior over the whole concentration range tested with regression coefficients $R^2 > 0.99$ for all compounds except dichlorvos ($R^2 = 0.9711$) and malathion ($R^2 = 0.9824$).

4.2.4 Sample analysis

Standard curves were obtained for all the analytes under investigation. The peak-area ratios (PARs) between compounds and IS (pentachlorobenzene) for each analyte were calculated and used to construct the standard curves (Figure 4.5 (a) – (h)). Equations and regression coefficient (R^2) of each standard curve were shown in the graphs.

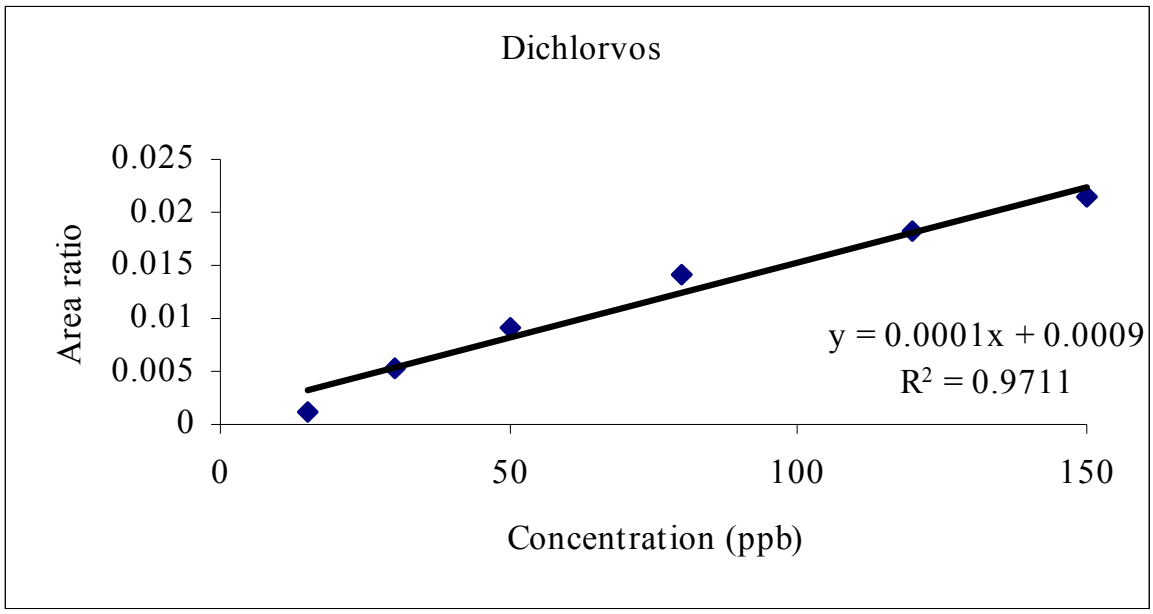


Figure 4.6 (a) Dichlorvos

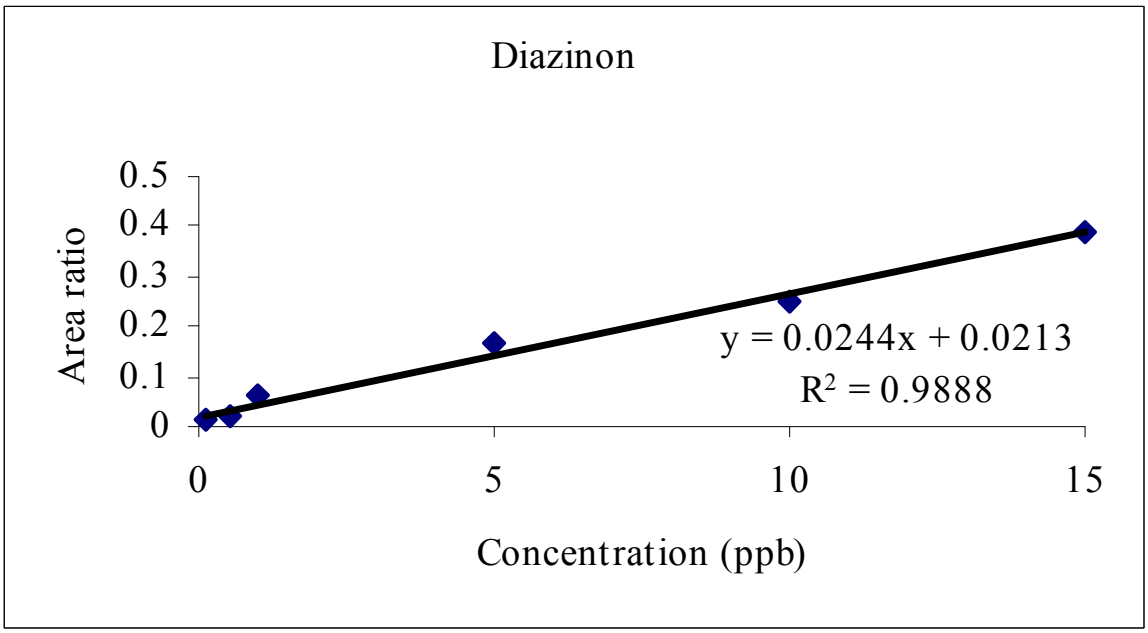


Figure 4.6 (b) Diazinon

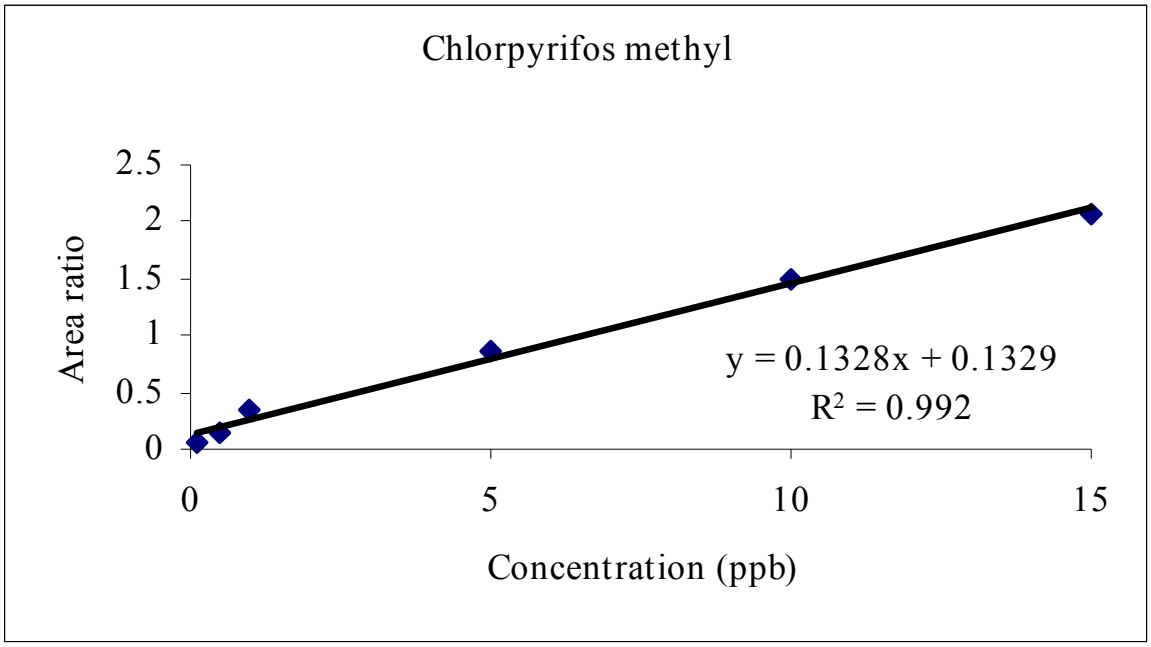


Figure 4.6 (c) Chlorpyrifos-methyl

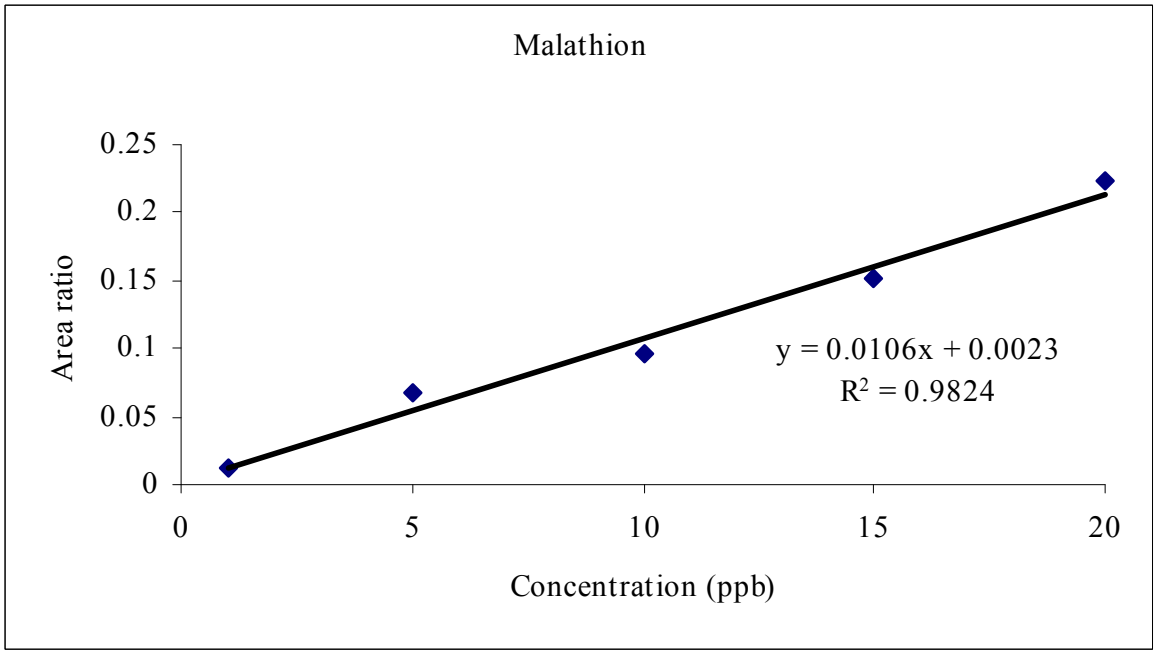


Figure 4.6 (d) Malathion

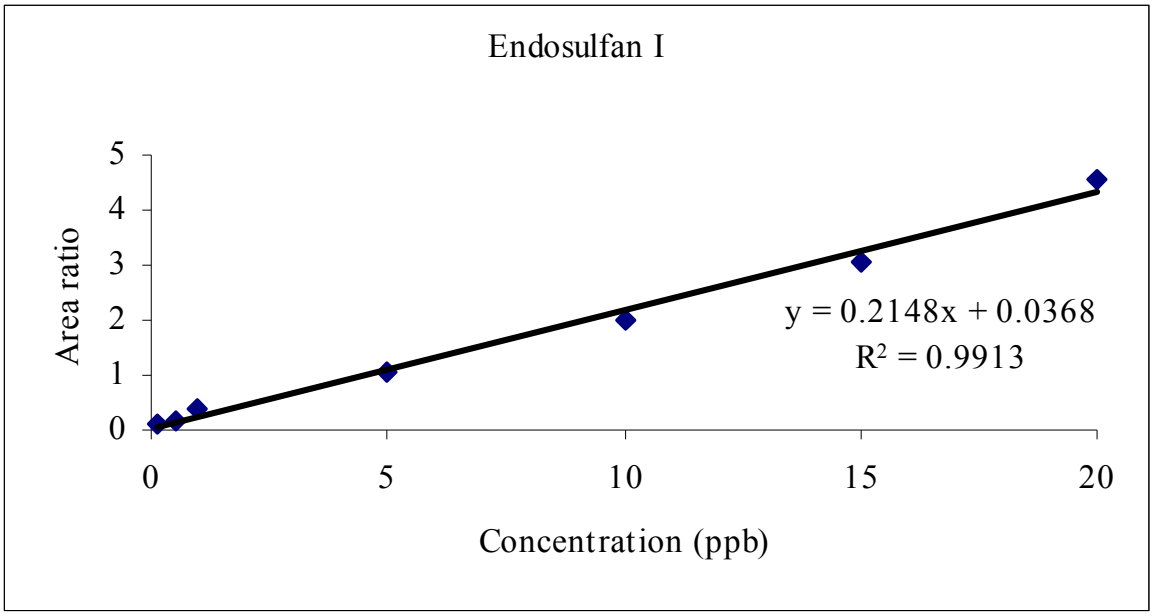


Figure 4.6 (e) Endosulfan I

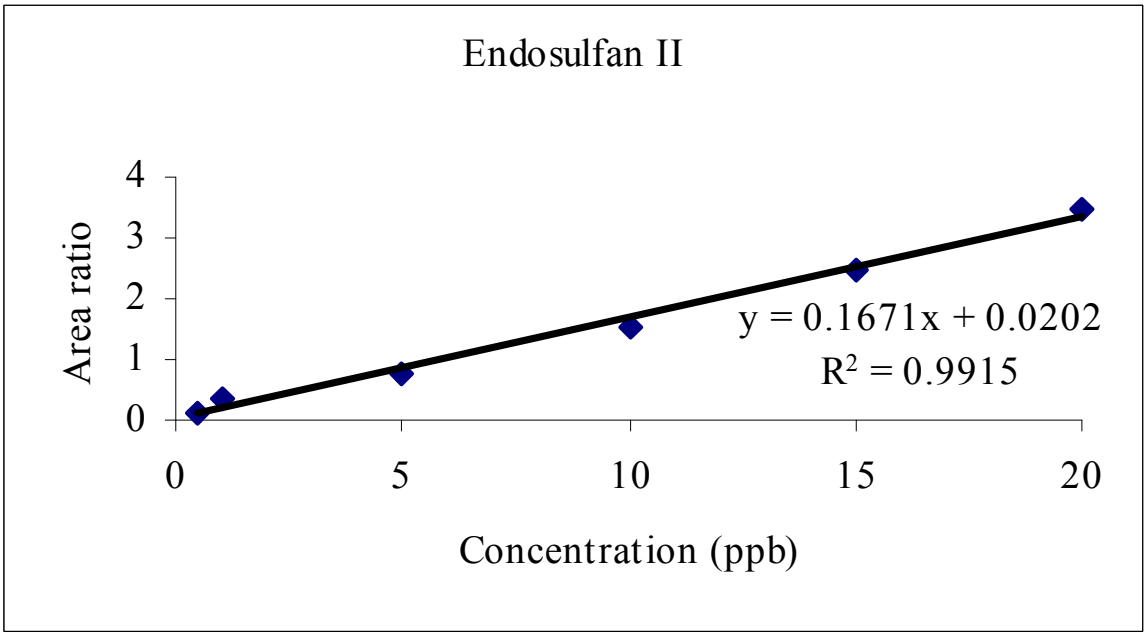


Figure 4.6 (f) Endosulfan II

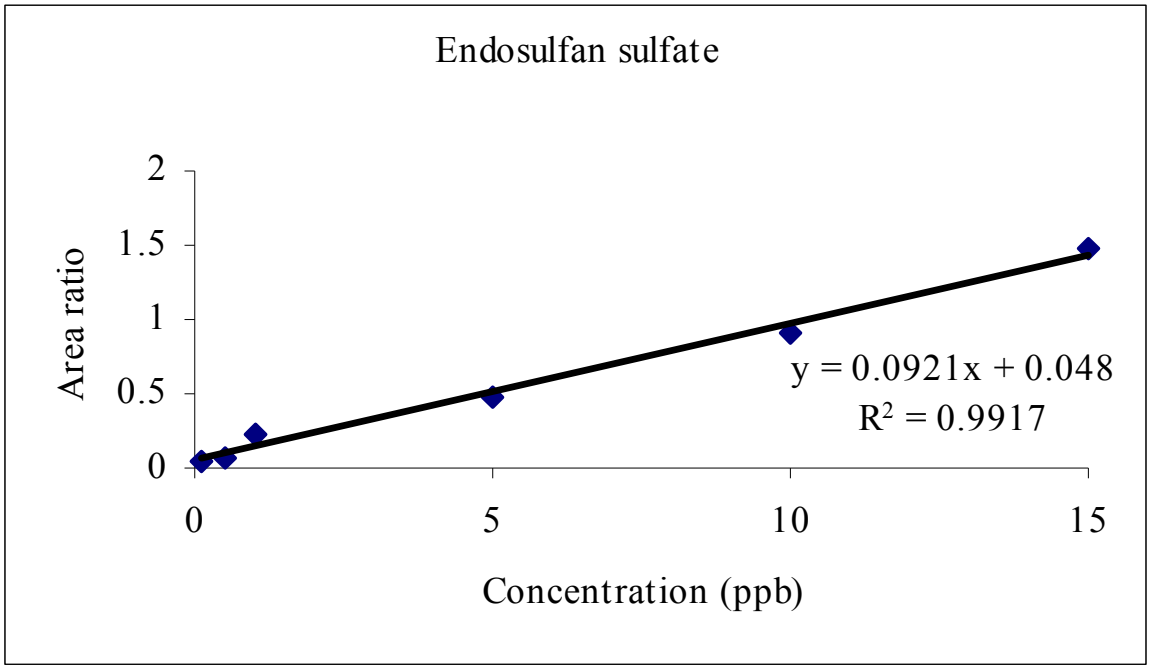


Figure 4.6 (g) Endosulfan sulfate

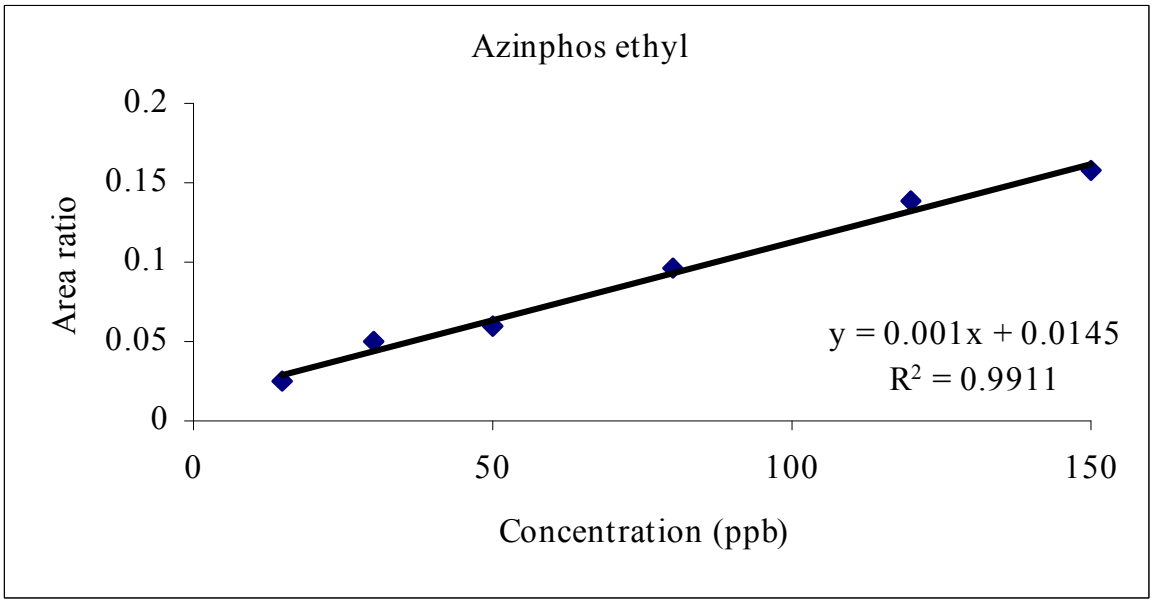
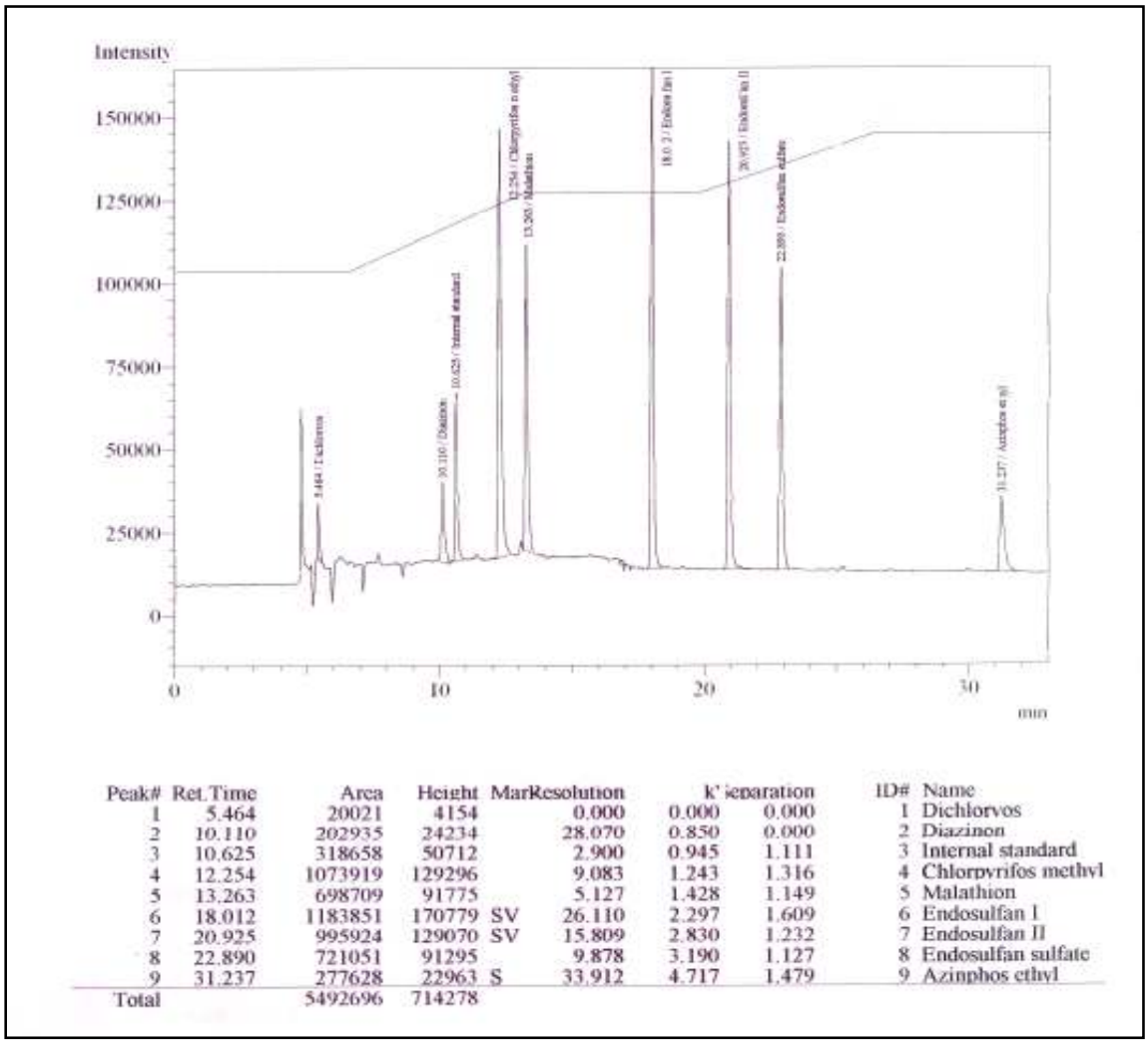


Figure 4.6 (h) Azinphos-ethyl

A representative chromatogram obtained following the injection of working standard mixture was shown in Figure 4.7.



Peak#	Ret.Time	Area	Height	MarResolution	k'	separation	ID#	Name
1	5.464	20021	4154	0.000	0.000	0.000	1	Dichlorvos
2	10.110	202935	24234	28.070	0.850	0.000	2	Diazinon
3	10.625	318658	50712	2.900	0.945	1.111	3	Internal standard
4	12.254	1073919	129296	9.083	1.243	1.316	4	Chlorpyrifos methyl
5	13.263	698709	91775	5.127	1.428	1.149	5	Malathion
6	18.012	1183851	170779	SV 26.110	2.297	1.609	6	Endosulfan I
7	20.925	995924	129070	SV 15.809	2.830	1.232	7	Endosulfan II
8	22.890	721051	91295	9.878	3.190	1.127	8	Endosulfan sulfate
9	31.237	277628	22963	S 33.912	4.717	1.479	9	Azinphos ethyl
Total		5492696	714278					

Figure 4.7 A representative chromatogram depicting that the separation of targeted analytes can be obtained using the GC-ECD assay described in Section 3.4.3.

Table 4.8 Concentrations of pesticides detected in water samples from ten sampling points in Manjung area

Sampling point	Dichlorvos			Diazinon			Chlorpyrifos methyl			Malathion		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1	nd	nd	nd	nd	nd	nd	nd	1.17	3.70	nd	nd	nd
2	nd	nd	84.35	0.32	nd	0.94	0.43	nd	0.90	nd	nd	1.50
3	nd	nd	nd	0.82	nd	nd	nd	nd	nd	4.10	nd	3.13
4	nd	nd	nd	2.59	nd	nd	nd	nd	0.82	7.21	3.07	nd
5	nd	nd	nd	nd	nd	0.78	0.97	0.92	0.47	6.99	nd	1.07
6	nd	nd	nd	nd	0.62	0.19	0.90	0.96	0.22	nd	nd	5.31
7	nd	nd	nd	1.02	0.19	1.93	0.86	1.42	1.37	nd	nd	4.34
8	nd	nd	nd	1.05	1.63	nd	0.81	1.27	0.90	nd	nd	15.04
9	nd	nd	nd	nd	1.20	3.65	0.85	0.70	0.29	nd	nd	0.14
10	nd	nd	88.50	nd	nd	0.54	0.81	1.08	2.13	nd	nd	14.51

Sampling point	Endosulfan I			Endosulfan II			Endosulfan sulfate			Azinphos ethyl		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1	nd	1.23	nd	nd	0.44	nd	nd	nd	nd	nd	nd	nd
2	0.90	1.03	0.48	nd	nd	0.45	nd	nd	0.84	nd	nd	nd
3	0.60	1.87	2.34	0.34	nd	nd	0.15	nd	nd	nd	nd	nd
4	nd	0.56	7.06	nd	0.24	3.30	nd	0.28	1.77	nd	nd	nd
5	1.59	0.51	0.93	nd	nd	0.13	nd	nd	nd	nd	nd	nd
6	1.31	0.92	2.18	1.45	1.52	2.77	0.45	nd	nd	nd	nd	79.61
7	0.93	0.29	0.13	0.68	0.41	0.25	0.78	nd	nd	nd	nd	nd
8	0.65	0.82	0.82	1.58	0.57	0.75	0.31	nd	0.52	nd	nd	nd
9	1.15	1.72	0.16	nd	1.36	6.61	nd	nd	5.26	nd	nd	55.95
10	1.08	1.71	1.24	1.68	1.84	0.57	0.40	1.05	0.12	nd	nd	64.03

1st: 27 May 2005; 2nd: 12 October 2005; 3rd: 8 December 2005

nd = not detected or below detection limit

Table 4.8 contains a summary of the occurrence and concentration of the targeted pesticides determined from the water samples collected in Manjung Straits during 27 May 2005 – 8 December 2005. Diazinon, chlorpyrifos methyl, malathion, endosulfan I, endosulfan II and endosulfan sulfate were compounds detected in the water samples. On the other hand, dichlorvos and azinphos ethyl were totally not detected.

Chlorpyrifos methyl, endosulfan I and endosulfan II were the most frequently detected pesticides. These compounds are the common insecticides applied in the aquaculture activity to kill organisms such as fish, crustaceans, snails, fungi, and algae (Gräslund and Bengtsson, 2001) during the pond treatment process. It is not surprising that detections of these compounds were observed in the surrounding water samples.

Endosulfan I has a maximum concentration of 7.06 ppb. Its occurrence was observed throughout the whole survey period with the exception of the 1st sampling expedition at sampling points 1 and 4, and 3rd sampling expedition at sampling point 1 with the exception of the degradation reduced concentrations to below the detection limit. Endosulfan II is another compound detected in most of the samples after endosulfan I. The maximum concentration detected for endosulfan II is 3.30 ppb. Endosulfan sulfate is also detected at maximum concentration of 5.26 ppb. Chlorpyrifos methyl was determined at almost all the sampling points throughout the 3 sampling expeditions, except point 3. The maximum concentration detected was 3.70 ppb.

Although occurrence of diazinon and malathion are not as abundant as the other compounds stated above, they are still detected in the water samples. The maximum concentrations detected were 3.65 ppb and 30.14 ppb, respectively.

The absences of dichlorvos and azinphos ethyl in the water samples may due to the occurrence of these compounds were below the method or instrumental detection limits. The sensitivity of the SPME method in detecting dichlorvos and azinphos ethyl was not as good as compare to the other compounds where the limits of detection (LODs) of these two compounds are 5 ppb.

4.3 Conclusion

SPME technique has been applied in a vast number of applications for the analysis of different type of pesticides in water samples. As a result, SPME could nearly be considered as a well established technique.

In this study, SPME using 100 μm PDMS fiber was evaluated for the analysis of pesticide residues like, diazinon, diclorvos, chlorpyrifos methyl, malathion, endosulfan I, endosulfan II, endosulfan sulfate and azinphos ethyl, in environmental water samples related to aquaculture activity. The effects of several parameters on SPME have been investigated. The optimum conditions for SPME were found to be operating at a temperature of 40˚C with an adsorption time of 30 min and at 270˚C for 12 min for the desorption process. The pH value and ionic strength of the sample need not to be adjusted before extraction process. The combination of

SPME with GC-ECD enables very low limit of detection to be achieved for the determination of both organochlorine and organophosphorus pesticides.

The SPME method used allows the determination of these compounds at very low concentrations where limits of detection (LODs) ranged from 0.01 – 5.00 ppb depending on the compound and the obtained recoveries were between 90 – 125% for all pesticides in the water samples. The described procedure is a sensitive, reproducible, simple, rapid and economical technique to rule out the presence of these pesticides in environmental waters at trace levels.

In conclusion, SPME with 100 μm PDMS coating is a precise and reproducible technique for both qualitative and quantitative determination of priority pesticide residues in environmental water samples. Optimization of the parameters affecting the method sensitivity should be carefully developed in order to maximize the amount extracted for most of the targeted analytes and to improve the limit of detection.

The impact of pesticides usage in the shrimp aquaculture activity towards adjacent water bodies in Sg. Manjung and its tributaries is difficult to conclude due to limited time and data obtained. However, the study area definitely deserved an extended and thorough investigation since the shrimp culture activities are expanding widely in parallel with the demand of the world market consumption.

The development of shrimp aquaculture activities has also caused the destruction of mangroves and wetlands which served both as nursing grounds for fishes and sea mammals as well as

buffers against occasional tidal wave. There is a need for further research to quantify the environmental effects of shrimp aquaculture towards ecological degradation of the mangrove forests.

REFERENCES

Aguilar, C., Penalver, S., Pocurull, E., Borrull, F. and Marcé, R.M. (1998). Solid-phase microextraction and gas chromatography with mass spectrometric detection for the determination of pesticides in aqueous samples. *Journal of Chromatography A* **795**: 105 – 115.

Ahmed, M. and Lorica, M.H. (2002). *Food Policy* **27**: 125 – 141.

Aksornkoae, S. (1993). *Ecology and Management of Mangroves*. Thailand: IUCN.

Alonso-Pérez, F., Ruiz-Luna, A., Turner, J., Berlanga-Robles, C.A., and Mitchelson-Jacob, G. (2003). Land cover changes and impact of shrimp aquaculture on the landscape in the Ceuta coastal lagoon system, Sinaloa, Mexico. *Ocean & Coastal Management* **46**: 583 – 600.

ASTM, (1997). *ASTM standards on environmental sampling* 2nd edn. West Conshohocken, PA.: American Society for Testing Materials.

Beltran, J., Lopez, F.J., Cepria, O., and Hernandez, F. (1998). Short communication: Solid-phase microextraction for quantitative analysis of organophosphorus pesticides in environmental water samples. *Journal of Chromatography A* **808**: 257 – 263.

Boussahel, R., Bouland, S., Moussaoui, K.M., Baudu, M. and Montiel, A. (2002). Technical note: Determination of chlorinated pesticides in water by SPME/GC. *Water Research* **36 (7)**: 1909 – 1911.

Boyd, C.E. and Massaut, L. (1999). Risk associated with the use of chemicals in pond aquaculture. *Aquaculture Engineering* **20**: 113 – 132.

Boyd, C.E. and Tucker, C.S. (1998). *Pond Aquaculture Water Quality Management*. Norwell MA: Kluwer.

Boyd, C.E. (1995). Chemistry and efficacy of amendments used to treat water and soil quality imbalance in shrimp ponds. In: Browdy, C.L. and Hopkins, J.S. (Eds.) *Swimming through troubled water*, Proceedings of the special session on shrimp farming, Aquaculture'95. Baton Rouge, Louisiana: World Aquaculture Society.

Boyd-Boland, A.A., Magdic, S. and Pawliszyn, J.B. (1996). Simultaneous Determination of 60 Pesticides in Water Using Solid-Phase Microextraction and Gas Chromatograph-Mass Spectrometry. *Analyst*, **121**: 929 – 938.

Braithwaite, A. and Smith, F.J. (1996). *Chromatographic Methods*, 5th edn. Blackie Academic and Professional, an imprint of UK: Chapman and Hall.

Brown, A.W.A. (1978). *Ecology of Pesticides*. New York: John Wiley and Sons.

Chee, K-K., Wong, M-K. and Lee, H-K. (1993). Sample preparation techniques for the analysis of organophosphorus pesticides in environmental samples: A review. *Sing. N. I. Chem*, **21**: 81 – 99.

Christian, G.D. (1994). *Analytical Chemistry*, 5th edn. New York: John Wiley and Sons, Inc.

Clesceri, L.S. *et. al.,* (1989). *Standard Methods: for the Examination of Water and Wastewater.* 17th edn. American Public Health Association.

Csuros, M. (1994). *Environmental Sampling and Analysis for Technicians*, Boca Raton, FL: Lewis Publishers Inc.

Dean, J.R. (1998). *Extraction Methods for Environmental Analysis*. Chinchester, UK: John Wiley and Sons.

Dewalt, B. R., Vergne, P. and Hardin, M. (1996). Shrimp Aquaculture Development and the Environment: People, Mangroves and Fisheries on the Gulf of Fonseca, Honduras. *World Development*, **24(7)**: 1193 – 1208.

DOF (2004). *Annual Fisheries Statistic 2004 (Volume 1).* Department of Fisheries, Ministry of Agricultural and Agro-based Industry, Malaysia.

Dong, C., Zeng, Z., and Yang, M. (2005). Determination of organochlorines pesticides and their derivations in water after HS-SPME using polymethylphenylvinylsiloxane-coated fiber by GC-ECD. *Water Research* **39**: 4204 – 4210.

Emden, H.F.V. and Peakall, D.B. (1996). *Beyond Silent Spring*. London: Chapman and Hall.

FAO (1995). *Code of Conduct for Responsible Fisheries*. Rome: FAO. In Bondad-Reabtaso, M. et al. (2005). Disease and health management in Asian aquaculture, *Veterinary Parasitology* **132**: 249 – 272.

FAO (1997). *FAO Technical Guidelines for Responsible Fisheries. No. 5.* Rome: FAO.

FAO (2002). *The state of world fisheries and aquaculture 2002.* Rome: FAO.

FAO (2004a). *The state of world fisheries and aquaculture 2004*. Rome: FAO.

FAO (2004b). *Capture-based aquaculture – The farming of eels, groupers, tunas and yellowtails.* Rome: FAO.

GESAMP (1997). IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP, Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. *Towards safe and effective use of chemicals in coastal aquaculture*. Reports and Studies, GESAMP. No. 65. Rome: FAO.

Gräslund, S. and Bengtsson, B.E. (2001). Chemicals and biological products used in south-east Asia shrimp farming, and their potential impact on the environment – A review. *The Science of the Total Environment* **280**: 93 – 131.

Gräslund, S., Holmström, K. and Wahlström, A. (2003). A field survey of chemicals and biological products used in shrimp farming. *Marine Pollution Bulletin* **46**: 81 – 90.

Green, J.M. (1996). A Practical Guide to Analytical Method Validation. *Analytical Chemisry* **68**: 305A – 309A.

Grob, R.L. (1995). *Modern Practice of Gas Chromatography*, 3rd edn. New York: John Wiley and Sons, Inc.

Harris, D.C. (2002). *Quantitative Chemical Analysis*, 6th edn. New York: W.H. Freeman and Company.

Hayes, W.J. and Laws, E.R. (1991). *Handbook of Pesticide Toxicologi.* (RA 1270 P4 Han).

Hutchinson T.H., Scholz N., and Guhl W. (1998). Analysis of the ECETOC Aquatic Toxicity (EAT) database' IV – comparative toxicity of chemical substances to freshwater vs saltwater organisms. *Chemosphere* **36**: 143 – 153.

INFOFISH International, (2001). February 2001.

Jeffery, G.H., Bassett, J., Mendham, J. and Denney, R.C. (1989). *Vogel's Textbook of Quantitative Chemical Analysis.* 5th edn. UK: Longman Scientific and Technical.

Jennings, S., Kaiser, M.J. and Reynolds, J.D. (2001). *Marine Fisheries Ecology*. London: Blackwell Science Ltd.

Jiménez, C., Ventura, R. and Segura, J. (2002). Validation of qualitative chromatographic methods: strategy in antidoping control laboratories. *Journal of Chromatography B* **767**: 341 – 351.

Joseph, M.M. (1990). *Aquaculture in Asia*. India: Asian Fisheries Society.

Krutz, L.J., Senseman, S.A. and Sciumbato, A.S. (2003). Review: solid-phase microextraction for hernicide determination in environmental samples. *Journal of Chromatography A* **999**: 103 – 121.

Lambropoulou, D.A., Sakkas, V.A. and Albanis, T.A. (2002). Validation of an SPME method, using PDMS, PA, PDMS-DVB, and CW-DVB SPME fiber coatings, for analysis of organophosphorus insecticides in natural waters. *Anal. Bioanal. Chem.*, **374:** 932 – 941.

Lambropoulou, D.A., and Albanis, T.A. (2001). Optimization of headspace solid-phase microextraction conditions for the determination of organophosphorus insecticides in natural waters. *Journal of Chromatography A* **922**: 243 – 255.

Lambropoulou, D.A., Konstantinou, I.K. and Albanis, T.A. (2000). Determination of fungicides in natural waters using solid-phase microextraction and gas chromatography coupled with electron-capture and mass spectrometric detection. *Journal of Chromatography A* **893**: 143 – 156.

Landau, M. (1992). *Introduction to Aquaculture*. USA: John Wiley and Sons, Inc.

Leight A.K. and Van Dolah R.F. (1999). Acute toxicity of the insecticides endosulfan, chlorpyrifos, and malathion to the epibenthic estuarine amphipod *Gammarus palustris* (Bousfield). *Environ. Toxicol. Chem.* **18**: 958 – 964.

Lenore S. Clesceri *et. al.,* (1998). *Standard methods for the examination of water and wastewater*, 20th edn. D.C: American Public Health Association.

Magdic, S. and Pawliszyn, J.B. (1996). Analysis of organochlorine pesticides using solid-phase microextraction. *Journal of Chromatography A* **723**: 111 – 122.

Magdic, S., Boyd-Boland, A., Jinno, K. and Pawliszyn, J.B. (1996). Analysis of organophosphorus insecticides from environmental samples using solid-phase microextraction. *Journal of Chromatography A* **736**: 219 – 228.

McEwen, F.L. and Stephenson, G.R. (1979). *The use and significance of pesticides in the environment*. New York: John Wiley and Sons.

Meyers, R.A. (2000). *Encyclopedia of Analytical Chemistry – Application, Theory and Instrumentation*. United Kingdom: John Wiley.

Mitra, S. (2003). *Sample Preparation Techniques in Analytical Chemistry*, New York: John Wiley and Sons, Inc.

Páez-Osuna, F. (2001a). The Environmental Impact of Shrimp Aquaculture: Causes, Effects, and Mitigating Alternatives. *Environmental Management*, **28 (1)**: 131 – 140.

Páez-Osuna, F. (2001b). The environmental impact of shrimp aquaculture: a global perspective. *Environmental Pollution* **112**: 229 – 231.

Páez-Osuna, F., Gracia, A., Flores-Verdugo, F., Lyle-Fritch, L.P., Alonson-Rodríguez, R., Roque, A., and Ruiz-Fernández, A.C. (2003). Review – Shrimp Aquaculture Development and the Environment in the Gulf of California Ecoregion. *Marine Pollution Bulletin* **46**: 806 – 815.

Page, B.D. and Lacroix, G. (1997). Application of solid-phase microextraction to the headspace gas chromatographic analysis of semi-volatile organochlorines contaminants in aqueous matrices. *Journal of Chromatography A* **757**: 173 – 182.

Pawliszyn, J.B. (1997). *Solid Phase Microextraction: Theory and Practice*. New York: John Wiley and Sons, Inc.

Phillips, M.J., Lin, C. K., and Beverage, M.C.M. (1993). Shrimp Culture and Environment: Lessons from the World's Most Rapidly Expanding Warmwater Aquaculture Sector. In: Pullin, R.S.V., Rosenthal, H. and Maclean, J.L. (Eds), *Environment and Aquaculture in Developing Countries.* pp 171 – 197. Philippines: ICLARM Conference Proceeding, 31, 359 pp.

Primavera, J.H. (1993). A critical review of shrimp pond culture in the Philippines, *Rev. Fish Sci.* **1**: 151 – 201.

Primavera, J.H. (1998). Mangroves as Nurseries: Shrimp Populations in Mangrove and Non-mangrove Habitats. *Estuarine, Coastal and Shelf Science* **46**: 457 – 464.

Primavera, J.H. (2005). Mangroves, fishponds, and the Quest for Sustainability. *Science*, **310**: 57 – 59.

Primavera, J.H., Lavilla-Pitogo, C.R., Ladja, L.M. and Dela Pena, M.R. (1993). A survey of chemical and biological products used in intensive shrimp farms in the Philippines. *Marine Pollution Bulletin* **26**: 35 – 40.

Richardson, M.L. (1992). *The dictionary of substances and their effects*, Vol. 7. Cambridge: The Royal Society of Chemistry.

Rönnbäck, P. (1999). Analysis: The ecological basis for economic value of seafood production supported by mangrove ecosystems. *Ecological Economics*, **29**: 235 – 252.

Rouessac, F. and Rouessac, A. (2000). *Chemical Analysis – Modern Instrumental Methods and Techniques*, English edn. Chichester, UK: John Wiley and Sons.

Sampedro, M.C., Martín, O., López de Armentia, C., Goicolea, M.A., Rodríguez, E, Gómez de Balugera, Z., Costa-Moreira, J. and Barrio, R.J. (2000). Solid-phase microextraction for the determination of systemic and non-volatile pesticides in river water using gas chromatography with nitrogen-phosphorous and electron-capture detection. *Journal of Chromatography A* **893**: 347 – 358.

Santos, F.J. and Galceran, M.T. (2002). The application of gas chromatography to environmental analysis. *Trends in Analytical Chemistry* **21 (9+10)**: 672 – 685.

Sauret-Szczepanski, N., Mirabel, P. and Wortham, H. (2006). Development of an SPME-GC-MS/MS method for the determination of pesticides in rainwater: Laboratory and field experiments. *Environmental Pollution* **139**: 133 – 142.

Selvaraj, O. (1998). Improving Management of Aquaculture in Asia: Report of an Asian Productivity Organization (APO) Seminar, 8 -16 May 1996. Asian Productivity Organization, Tokyo, 1998.

Serrano, P.H. (2005). *Responsible use of antibiotics in aquaculture*. FAO, Fisheries Technical Paper No. 469. Rome: FAO.

Shang, Y.C. Leung, P. and Ling, B-H. (1998). Comparative economics of shrimp farming in Asia. *Aquaculture*, **164**: 183 – 200.

Shimadzu (1995). *Gas Chromatograph GC 17-A Ver. 2 User's Manual*. Japan: Shimadzu Corporation.

Skoog, D.A., West, D.M. and Holler, F.J. (1994). *Analytical Chemistry – An Introduction.* 6th edn. USA: Saunders College Publishing.

Stickney, R.R. (1994). *Principles of Aquaculture*. USA: John Wiley and Sons, Inc., USA.

Subasinghe, R.P., Barg, U. and Tacon, A. (1996). Chemicals in Asian Aquaculture: Need, Usage, Issues and Challenges. pp 1 – 4. In Arthur, J.R. *et. al.* (eds). *Use of Chemicals in Aquaculture in Asia*, Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia, 20 – 22 May 1996, Tigbauan, Iloilo, Philippines.

Tacon, A.G.J., and Forster, I.P. (2003). Aquafeeds and the environment: policy implications. *Aquaculture*, **226**: 181 – 189.

Tovar, A., Moreno, C., Manuel-Vez, M.P. and García-Vargas, M. (2000). Environmental Impacts of Intensive Aquaculture in Marine Waters. *Water Research* **34(1)**: 334 – 342.

Valor, I., Moltó, J.C., Apraiz, D. and Font, G. (1997). Matrix effects on solid-phase microextraction of organophosphorus pesticides from water. *Journal of Chromatography A* **767**: 195 – 203.

Verschueren, K. (2001). *Handbook of Environmental Data on Organic Chemicals*, *Vol. 1.* 4th edn. New York: John Wiley and Sons. Inc.

Wercinski, S.A.S. (1999). *Solid Phase Microextraction – A Practical Guide*. New York: Marcel Dekker, Inc.

Zhang, Z and Pawliszyn, J.B. (1995). *Anal. Chem.*, **67**: 34. In Valor, I., Moltó, J.C., Apraiz, D. And Font, G. (1997). Matrix effects on solid-phase microextraction of organophosphorus pesticides from water. *Journal of Chromatography A* **767**: 195 – 203.

APPENDIX

1. Raw Data for Method Validation

1.1 Dichlorvos

Solution 1										
	1	2	3	4	5	6	7	Average	SD	RSD
Dichlorvos	558	598	659	603	720	543	572	607.5714286	62	10%
IS	324328	275113	193791	265234	165708	122975	199419	220938	70040	32%
Ratio	0.001720	0.002174	0.00340057	0.002273	0.004344992	0.004415532	0.002868	0.003028	0.001067	35%

Solution 2										
	1	2	3	4	5	6	7	Average	SD	RSD
Dichlorvos	1487	1411	1109	1162	1116	1143	1156	1226	155	13%
IS	375473	328473	251572	151358	204134	194321	181744	241011	82595	34%
Ratio	0.003960	0.004296	0.004408	0.007677	0.005467	0.005882	0.006361	0.005436	0.001330	24%

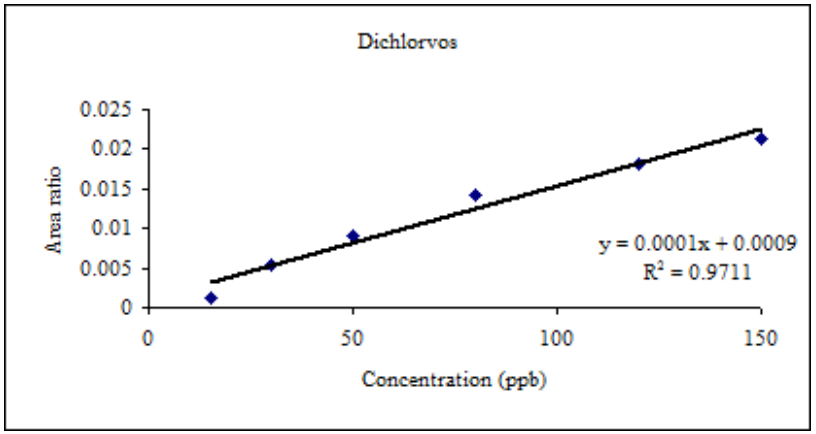
Solution 3										
	1	2	3	4	5	6	7	Average	SD	RSD
Dichlorvos	1314	1508	1677	2021	963	1032	1014	1361	397	29%
IS	280047	199472	176727	169305	86551	119321	97168	161227	67482	42%
Ratio	0.004692	0.007560	0.009489	0.011937	0.011126	0.008649	0.010436	0.009127	0.002453	27%

Solution 4										
	1	2	3	4	5	6	7	Average	SD	RSD
Dichlorvos	3799	3554	3252	3228	2027	3038	2084	2997	689	23%
IS	385181	301332	244382	216040	137237	160547	136468	225884	92763	41%
Ratio	0.009863	0.011794	0.013307	0.014942	0.014770	0.018923	0.015271	0.014124	0.002878	20%

Solution 5										
	1	2	3	4	5	6	7	Average	SD	RSD
Dichlorvos	6847	6256	4957	4297	5247	4934	4810	5335	894	17%
IS	502713	377884	289441	312056	306872	252452	164849	315181	105179	33%
Ratio	0.013620	0.016555	0.017126	0.013770	0.017098	0.019544	0.029178	0.018127	0.005291	29%

Solution 6										
	1	2	3	4	5	6	7	Average	SD	RSD
Dichlorvos	7766	6479	7439	7302	6047	6600	6371	6858	641	9%
IS	553843	338134	419041	296629	268196	266924	240341	340444	111271	33%
Ratio	0.014022	0.019161	0.017752	0.024617	0.022547	0.024726	0.026508	0.021333	0.004501	21%

	15	30	50	80	120	150
Dichlorvos	0.003028	0.005436	0.009127	0.014124	0.018127	0.021333



1.2 Diazinon

Solution 1										
	1	2	3	4	5	6	7	Average	SD	RSD
Diazinon	3104	2917	2275	2996	2108	1605	2341	2478	550	22%
IS	324328	275113	193791	265234	165708	122975	199419	220938	70040	32%
Ratio	0.009571	0.010603	0.011739	0.011296	0.012721	0.013051	0.011739	0.011531	0.001195	10%

Solution 2										
	1	2	3	4	5	6	7	Average	SD	RSD
Diazinon	6336	6292	5118	3490	4488	4262	3920	4844	1122	23%
IS	375473	328473	251572	151358	204134	194321	181744	241011	82595	34%
Ratio	0.016875	0.019155	0.020344	0.023058	0.021986	0.021933	0.021569	0.020703	0.002107	10%

Solution 3										
	1	2	3	4	5	6	7	Average	SD	RSD
Diazinon	12680	11282	10420	10361	6229	7923	6161	9294	2547	27%
IS	280047	199472	176727	169305	86551	119321	97168	161227	67482	42%
Ratio	0.045278	0.056559	0.058961	0.061197	0.071969	0.066401	0.063406	0.060539	0.008415	14%

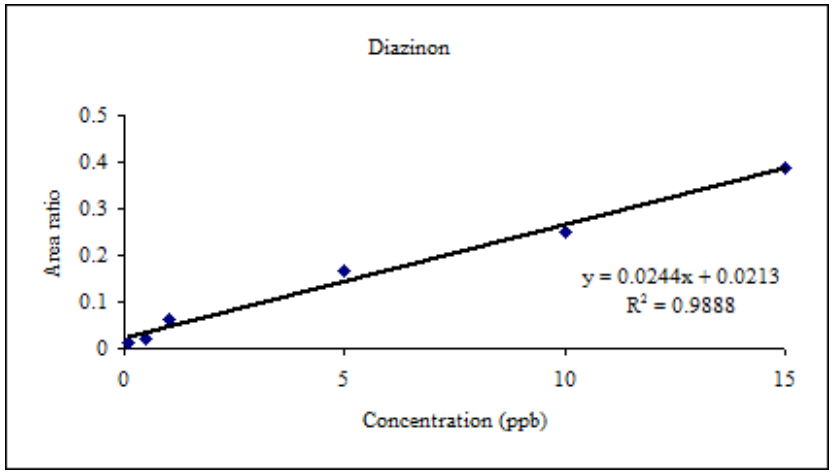
Solution 4										
	1	2	3	4	5	6	7	Average	SD	RSD
Diazinon	50726	43774	40948	36300	25668	29723	24248	35912	9883	28%
IS	385181	301332	244382	216040	137237	160547	136468	225884	92763	41%
Ratio	0.131694	0.145268	0.167557	0.168024	0.187034	0.185136	0.177683	0.166057	0.020649	12%

Solution 5										
	1	2	3	4	5	6	7	Average	SD	RSD
Diazinon	107799	90998	75769	73643	73781	65970	50381	76906	18248	24%
IS	502713	377884	289441	312056	306872	252452	164849	315181	105179	33%
Ratio	0.214434	0.240809	0.261777	0.235993	0.240429	0.261317	0.305619	0.251483	0.028816	11%

Solution 6										
	1	2	3	4	5	6	7	Average	SD	RSD
Diazinon	161378	130193	138702	121566	115741	115219	105197	126857	18705	15%
IS	553843	338134	419041	296629	268196	266924	240341	340444	111271	33%
Ratio	0.291379	0.385034	0.330999	0.409825	0.431554	0.431655	0.437699	0.388306	0.056777	15%

Solution 7										
	1	2	3	4	5	6	7	Average	SD	RSD
Diazinon	177030	167394	157798	150153	141416	123591	119802	148169	21423	14%
IS	391159	291016	199041	170069	187239	146989	169492	222144	87744	39%
Ratio	0.452578	0.575205	0.792791	0.882895	0.755270	0.840818	0.706830	0.715198	0.152843	21%

	0.1	0.5	1	5	10	15	20
Diazinon	0.011531	0.020703	0.060539	0.166057	0.251483	0.388306	0.715198



1.3 Chlorpyrifos methyl

Solution 1										
	1	2	3	4	5	6	7	Average	SD	RSD
Chlorpyrifos methyl	18630	17751	13381	17112	12532	9010	13456	14553	3435	24%
IS	324328	275113	193791	265234	165708	122975	199419	220938	70040	32%
Ratio	0.057442	0.064523	0.069049	0.064517	0.075627	0.073267	0.067476	0.067414	0.006063	9%

Solution 2										
	1	2	3	4	5	6	7	Average	SD	RSD
Chlorpyrifos methyl	43320	42412	34816	23659	28637	25641	25271	31965	8277	26%
IS	375473	328473	251572	151358	204134	194321	181744	241011	82595	34%
Ratio	0.115374	0.129119	0.138394	0.156312	0.140285	0.131952	0.139047	0.135783	0.012482	9%

Solution 3										
	1	2	3	4	5	6	7	Average	SD	RSD
Chlorpyrifos methyl	77501	68237	62204	58964	36107	44342	33098	54350	16815	31%
IS	280047	199472	176727	169305	86551	119321	97168	161227	67482	42%
Ratio	0.276743	0.342088	0.351978	0.348271	0.417176	0.371619	0.340627	0.349786	0.041840	12%

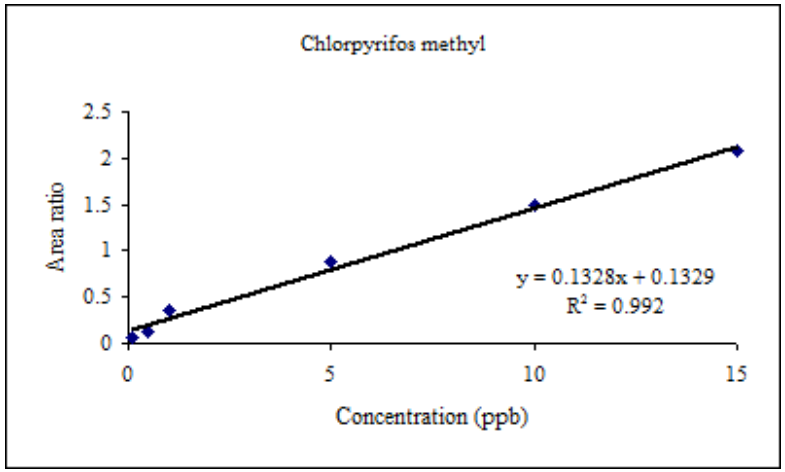
Solution 4										
	1	2	3	4	5	6	7	Average	SD	RSD
Chlorpyrifos methyl	283142	244255	211755	193173	136650	150372	122280	191661	59281	31%
IS	385181	301332	244382	216040	137237	160547	136468	225884	92763	41%
Ratio	0.735088	0.810584	0.866492	0.894154	0.995723	0.936623	0.896034	0.876385	0.084621	10%

Solution 5										
	1	2	3	4	5	6	7	Average	SD	RSD
Chlorpyrifos methyl	665011	570846	466856	432506	417295	367316	287293	458160	126054	28%
IS	502713	377884	289441	312056	306872	252452	164849	315181	105179	33%
Ratio	1.322844	1.510638	1.612957	1.385988	1.359834	1.454993	1.742765	1.484289	0.150629	10%

Solution 6										
	1	2	3	4	5	6	7	Average	SD	RSD
Chlorpyrifos methyl	970340	755028	790919	657260	589253	576976	506125	692272	158521	23%
IS	553843	338134	419041	296629	268196	266924	240341	340444	111271	33%
Ratio	1.752013	2.232925	1.887450	2.215764	2.197098	2.161574	2.105862	2.078955	0.185965	9%

Solution 7										
	1	2	3	4	5	6	7	Average	SD	RSD
Chlorpyrifos methyl	1028989	951271	857681	792426	730797	614883	589184	795033	164333	21%
IS	391159	291016	199041	170069	187239	146989	169492	222144	87744	39%
Ratio	2.630616	3.268793	4.309067	4.659438	3.903017	4.183191	3.476176	3.775757	0.695895	18%

	0.1	0.5	1	5	10	15	20
Chlorpyrifos methyl	0.067414	0.135783	0.349786	0.876385	1.484289	2.078955	3.775757



1.4 Malathion

Solution 1										
	1	2	3	4	5	6	7	Average	SD	RSD
Malathion	0	0	0	0	0	0	0	0	0	#DIV/0!
IS	324328	275113	193791	265234	165708	122975	199419	220938	70040	32%
Ratio	0	0	0	0	0	0	0	0	0	#DIV/0!

Solution 2										
	1	2	3	4	5	6	7	Average	SD	RSD
Malathion	0	0	0	0	0	0	0	0	0	#DIV/0!
IS	375473	328473	251572	151358	204134	194321	181744	241011	82595	34%
Ratio	0	0	0	0	0	0	0	0	0	#DIV/0!

Solution 3										
	1	2	3	4	5	6	7	Average	SD	RSD
Malathion	1744	1809	1820	2053	1360	1686	1382	1693	248	15%
IS	280047	199472	176727	169305	86551	119321	97168	161227	67482	42%
Ratio	0.006228	0.009069	0.010298	0.012126	0.015713	0.014130	0.014223	0.011684	0.003351	29%

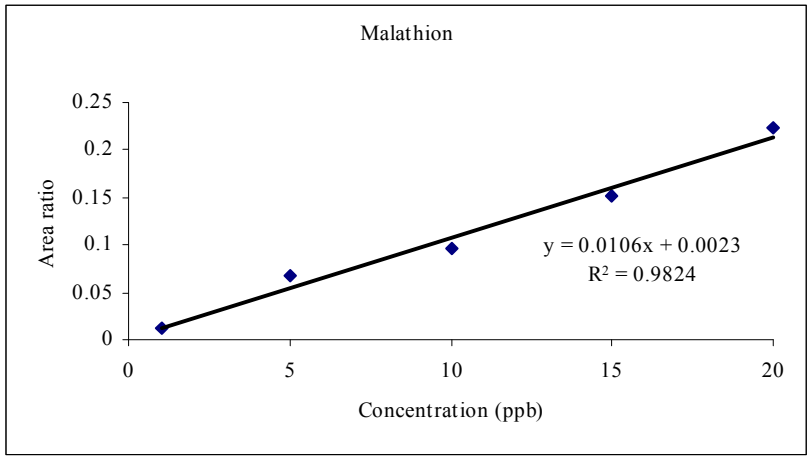
Solution 4										
	1	2	3	4	5	6	7	Average	SD	RSD
Malathion	15729	14737	14436	14502	11388	14108	11530	13776	1661	12%
IS	385181	301332	244382	216040	137237	160547	136468	225884	92763	41%
Ratio	0.040835	0.048906	0.059071	0.067126	0.082981	0.087875	0.084489	0.067326	0.018579	28%

Solution 5										
	1	2	3	4	5	6	7	Average	SD	RSD
Malathion	30086	29396	27452	26320	27879	27377	27132	27949	1327	5%
IS	502713	377884	289441	312056	306872	252452	164849	315181	105179	33%
Ratio	0.059847	0.077791	0.094845	0.084344	0.090849	0.108444	0.164587	0.097244	0.033293	34%

Solution 6										
	1	2	3	4	5	6	7	Average	SD	RSD
Malathion	49739	47771	47736	47484	48436	47627	46858	47950	915	2%
IS	553843	338134	419041	296629	268196	266924	240341	340444	111271	33%
Ratio	0.089807	0.141278	0.113917	0.160079	0.180599	0.178429	0.194965	0.151296	0.038422	25%

Solution 7										
	1	2	3	4	5	6	7	Average	SD	RSD
Malathion	46441	47815	45809	46981	44470	43613	41436	45224	2204	5%
IS	391159	291016	199041	170069	187239	146989	169492	222144	87744	39%
Ratio	0.118727	0.164304	0.230149	0.276247	0.237504	0.296709	0.244472	0.224016	0.062319	28%

	1	5	10	15	20
Malathion	0.011684	0.067326	0.097244	0.151296	0.224016



1.5 Endosulfan I

Solution 1										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan I	35488	30096	23685	28690	18101	14188	20386	24376	7465	31%
IS	324328	275113	193791	265234	165708	122975	199419	220938	70040	32%
Ratio	0.109420	0.109395	0.122219	0.108169	0.109234	0.115373	0.102227	0.110862	0.006299	6%

Solution 2										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan I	49668	52417	40196	26607	32555	30265	28288	37142	10466	28%
IS	375473	328473	251572	151358	204134	194321	181744	241011	82595	34%
Ratio	0.132281	0.159578	0.159779	0.175789	0.159479	0.155747	0.155648	0.156900	0.012831	8%

Solution 3										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan I	89068	75060	67062	60882	37678	43928	36636	58616	20048	34%
IS	280047	199472	176727	169305	86551	119321	97168	161227	67482	42%
Ratio	0.318047	0.376293	0.379467	0.359600	0.435327	0.368150	0.377038	0.373417	0.034582	9%

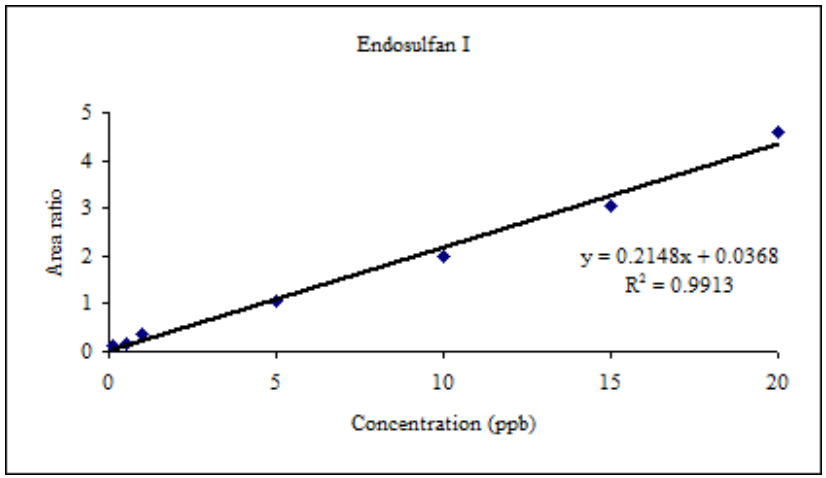
Solution 4										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan I	381292	302810	253953	231734	163265	160076	149873	234715	85999	37%
IS	385181	301332	244382	216040	137237	160547	136468	225884	92763	41%
Ratio	0.989903	1.004905	1.039164	1.072644	1.189657	0.997066	1.098228	1.055938	0.071485	7%

Solution 5										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan I	965617	736409	575703	659117	578862	468101	379699	623358	191017	31%
IS	502713	377884	289441	312056	306872	252452	164849	315181	105179	33%
Ratio	1.920812	1.948770	1.989017	2.112175	1.886330	1.854218	2.303314	2.002091	0.156919	8%

Solution 6										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan I	1516114	1004307	1276246	891489	976634	834481	705295	1029224	277877	27%
IS	553843	338134	419041	296629	268196	266924	240341	340444	111271	33%
Ratio	2.737444	2.970145	3.045635	3.005401	3.641494	3.126287	2.934560	3.065852	0.280870	9%

Solution 7										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan I	937897	1019318	938652	1021106	1037879	750123	806631	930229	112271	12%
IS	391159	291016	199041	170069	187239	146989	169492	222144	87744	39%
Ratio	2.397739	3.502618	4.715873	6.004069	5.543071	5.103259	4.759110	4.575105	1.238458	27%

	0.1	0.5	1	5	10	15	20
Endo I	0.110862	0.1569	0.373417	1.055938	2.002091	3.065852	4.575105



1.6 Endosulfan II

Solution 1										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan II	24373	23073	18784	21152	16168	12587	16398	18934	4204	22%
IS	324328	275113	193791	265234	165708	122975	199419	220938	70040	32%
Ratio	0.075149	0.083867	0.096929	0.079748	0.097569	0.102354	0.082229	0.088264	0.010492	12%

Solution 2										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan II	30452	34649	28305	20850	20738	19861	17662	24645	6436	26%
IS	375473	328473	251572	151358	204134	194321	181744	241011	82595	34%
Ratio	0.081103	0.105485	0.112513	0.137753	0.101590	0.102207	0.097181	0.105404	0.017224	16%

Solution 3										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan II	81693	75309	69655	64074	45068	50414	1127	55334	27213	49%
IS	280047	199472	176727	169305	86551	119321	97168	161227	67482	42%
Ratio	0.291712	0.377542	0.394139	0.378453	0.520710	0.422507	0.011598	0.342380	0.160946	47%

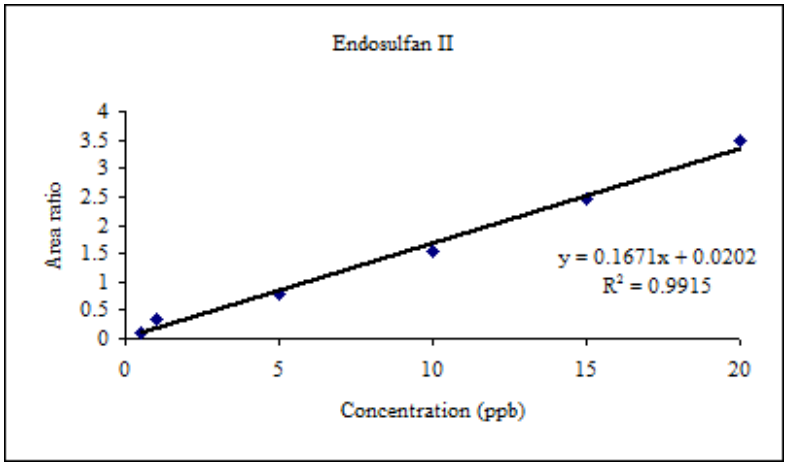
Solution 4										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan II	223901	197858	178300	168429	131440	133745	120431	164872	38356	23%
IS	385181	301332	244382	216040	137237	160547	136468	225884	92763	41%
Ratio	0.581288	0.656611	0.729595	0.779620	0.957759	0.833058	0.882485	0.774345	0.130349	17%

Solution 5										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan II	600047	528065	451377	477064	443243	401718	351559	464725	81540	18%
IS	502713	377884	289441	312056	306872	252452	164849	315181	105179	33%
Ratio	1.193617	1.397426	1.559478	1.528777	1.444390	1.591265	2.132612	1.549652	0.289574	19%

Solution 6										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan II	1035084	841412	908908	777698	695470	724745	659022	806048	132631	16%
IS	553843	338134	419041	296629	268196	266924	240341	340444	111271	33%
Ratio	1.868912	2.488398	2.169019	2.621787	2.593141	2.715174	2.742029	2.456923	0.322322	13%

Solution 7										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan II	851231	806290	793230	750439	715438	578405	572366	723914	110087	15%
IS	391159	291016	199041	170069	187239	146989	169492	222144	87744	39%
Ratio	2.176176	2.770604	3.985259	4.412556	3.820988	3.935022	3.376950	3.496794	0.781037	22%

	0.1	0.5	1	5	10	15	20
Endo II	0.088264	0.105404	0.34238	0.774345	1.549652	2.456923	3.496794



1.7 Endosulfan sulfate

Solution 1										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan sulfate	9074	9127	8219	8675	8813	6741	8214	8409	821	10%
IS	324328	275113	193791	265234	165708	122975	199419	220938	70040	32%
Ratio	0.027978	0.033175	0.042412	0.032707	0.053184	0.054816	0.041190	0.040780	0.010333	25%

Solution 2										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan sulfate	17918	18742	17558	15161	16230	16094	15529	16747	1336	8%
IS	375473	328473	251572	151358	204134	194321	181744	241011	82595	34%
Ratio	0.047721	0.057058	0.069793	0.100166	0.079507	0.082822	0.085444	0.074644	0.017871	24%

Solution 3										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan sulfate	36401	34736	34135	34896	27568	29654	26388	31968	4009	13%
IS	280047	199472	176727	169305	86551	119321	97168	161227	67482	42%
Ratio	0.129982	0.174140	0.193151	0.206113	0.318517	0.248523	0.271571	0.220285	0.063637	29%

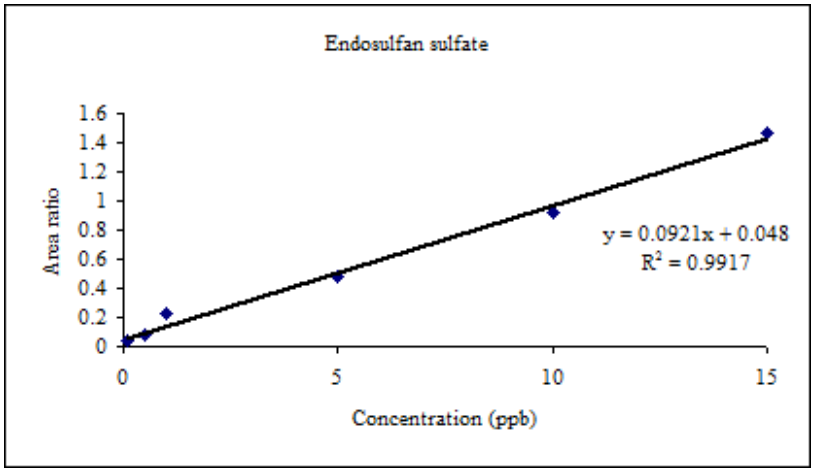
Solution 4										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan sulfate	114853	108082	102230	99739	85814	91746	82500	97852	11792	12%
IS	385181	301332	244382	216040	137237	160547	136468	225884	92763	41%
Ratio	0.298179	0.358681	0.418320	0.461669	0.625298	0.571459	0.604537	0.476878	0.127040	27%

Solution 5										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan sulfate	305586	292036	271264	265657	258197	247748	231198	267384	25379	9%
IS	502713	377884	289441	312056	306872	252452	164849	315181	105179	33%
Ratio	0.607874	0.772819	0.937200	0.851312	0.841383	0.981367	1.402483	0.913491	0.247164	27%

Solution 6										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan sulfate	532579	492421	481960	464712	449310	454454	432555	472570	33205	7%
IS	553843	338134	419041	296629	268196	266924	240341	340444	111271	33%
Ratio	0.961606	1.456290	1.150150	1.566644	1.675305	1.702560	1.799755	1.473187	0.309623	21%

Solution 7										
	1	2	3	4	5	6	7	Average	SD	RSD
Endosulfan sulfate	606396	599199	577503	570435	546179	521980	503134	560689	38668	7%
IS	391159	291016	199041	170069	187239	146989	169492	222144	87744	39%
Ratio	1.550255	2.058990	2.901427	3.354139	2.917015	3.551150	2.968482	2.757351	0.709682	26%

	0.1	0.5	1	5	10	15	20
Endosulfate	0.04078	0.074644	0.220285	0.476878	0.913491	1.473187	2.757351



1.8 Azinphos ethyl

Solution 1										
	1	2	3	4	5	6	7	10	SD	RSD
Azinphos ethyl	5123	5829	5299	5550	5375	4018	3973	5024	736	15%
IS	324328	275113	193791	265234	165708	122975	199419	220938	70040	32%
Ratio	0.015796	0.021188	0.027344	0.020925	0.032437	0.032673	0.019923	0.024326	0.006559	27%

Solution 2										
	1	2	3	4	5	6	7	35	SD	RSD
Azinphos ethyl	12002	12763	13129	12233	13376	14947	16642	13585	1656	12%
IS	502713	377884	289441	312056	306872	252452	164849	315181	105179	33%
Ratio	0.023874	0.033775	0.045360	0.039201	0.043588	0.059207	0.100953	0.049423	0.025178	51%

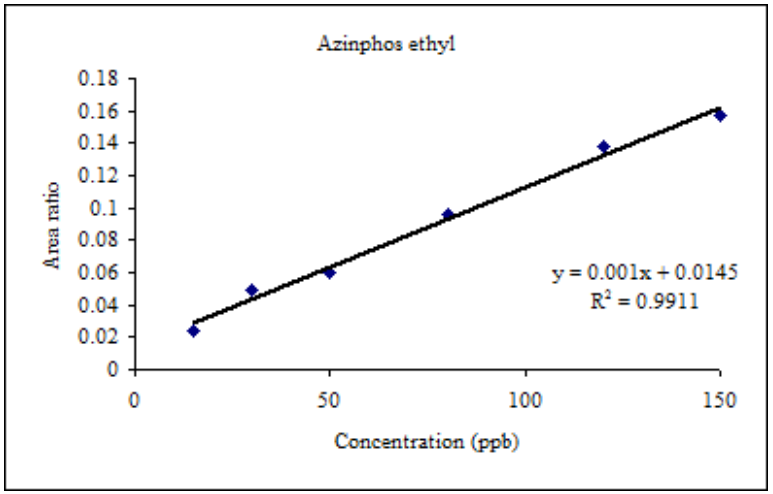
Solution 3										
	1	2	3	4	5	6	7	15	SD	RSD
Azinphos ethyl	13470	13610	13533	12534	13230	13439	13711	13361	394	3%
IS	375473	328473	251572	151358	204134	194321	181744	241011	82595	34%
Ratio	0.035875	0.041434	0.053794	0.082810	0.064810	0.069159	0.075441	0.060475	0.017461	29%

Solution 4										
	1	2	3	4	5	6	7	40	SD	RSD
Azinphos ethyl	25100	26761	27472	30154	28967	32591	36245	29613	3800	13%
IS	553843	338134	419041	296629	268196	266924	240341	340444	111271	33%
Ratio	0.045320	0.079143	0.065559	0.101656	0.108007	0.122098	0.150807	0.096084	0.035684	37%

Solution 5										
	1	2	3	4	5	6	7	30	SD	RSD
Azinphos ethyl	28689	27837	28481	28681	25940	27735	25444	27544	1328	5%
IS	385181	301332	244382	216040	137237	160547	136468	225884	92763	41%
Ratio	0.074482	0.092380	0.116543	0.132758	0.189016	0.172753	0.186447	0.137768	0.046117	33%

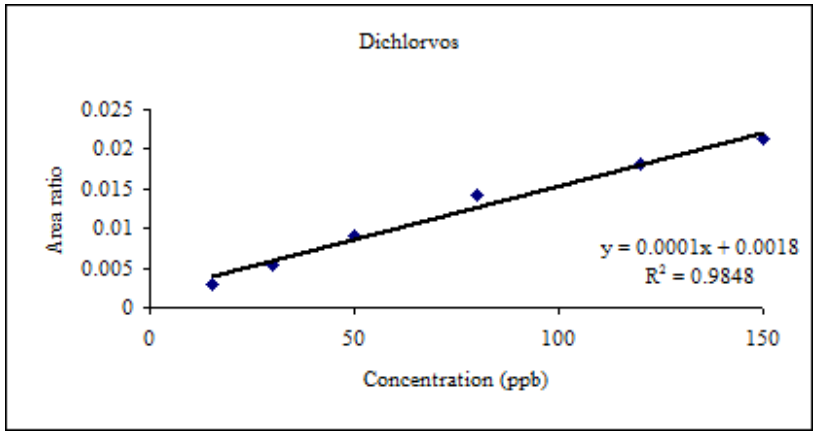
Solution 6										
	1	2	3	4	5	6	7	25	SD	RSD
Azinphos ethyl	24222	23675	24088	22918	19894	21977	20471	22464	1742	8%
IS	280047	199472	176727	169305	86551	119321	97168	161227	67482	42%
Ratio	0.086493	0.118688	0.136301	0.135365	0.229853	0.184184	0.210676	0.157366	0.052061	33%

	15	30	50	80	120	150
azinphos	0.024326	0.049423	0.060475	0.096084	0.137768	0.157366



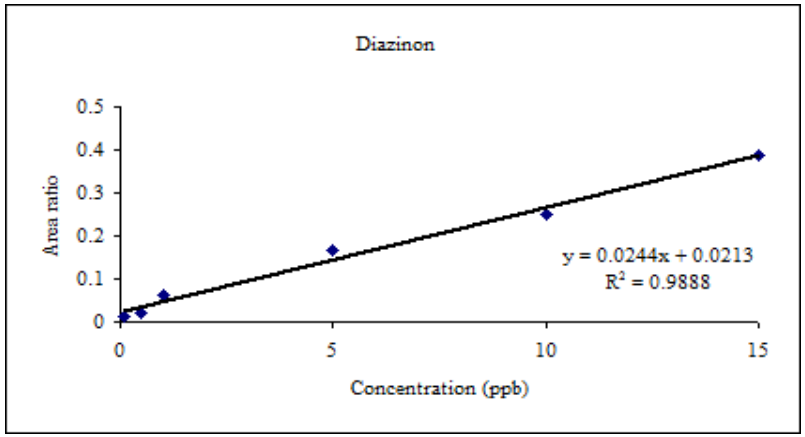
2. Raw Data for Sample Analysis

2.1 Dichlorvos



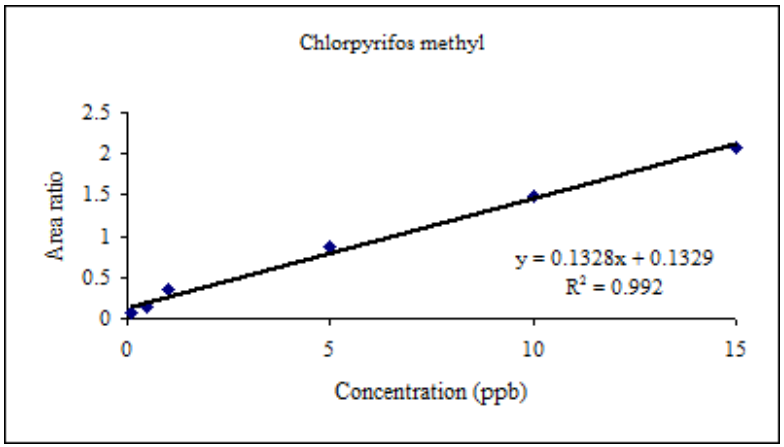
Sampling point	Average area ratio		
	27/05/05	12/10/05	8/12/05
1	0.001064	0.001102	0.001358
2	0.001113	0.001291	0.009335
3	0.001280	0.000568	0.001185
4	0.001213	0.001243	0.001076
5	0.000540	0.001343	0.001234
6	0.001269	0.001201	0.001125
7	0.000554	0.001164	0.001331
8	0.000216	0.001347	0.001210
9	0.001394	0.001237	0.001359
10	0.001066	0.000184	0.009750

2.2 Diazinon



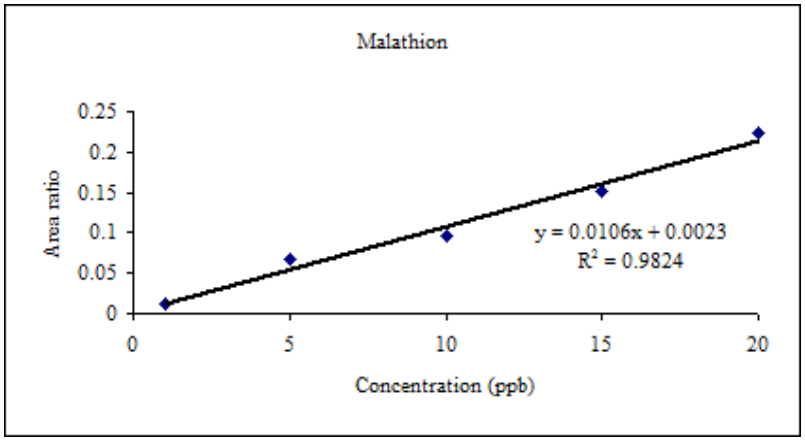
Sampling point	Average area ratio		
	27/05/05	12/10/05	8/12/05
1	0.014364	0.021346	0.020543
2	0.029108	0.021520	0.052969
3	0.041308	0.021432	0.021415
4	0.084499	0.021373	0.021035
5	0.013167	0.016437	0.048162
6	0.015220	0.036428	0.063356
7	0.046188	0.025936	0.063209
8	0.046920	0.061072	0.017225
9	0.021242	0.050580	0.052100
10	0.021490	0.016353	0.057655

2.3 Chlorpyrifos Methyl



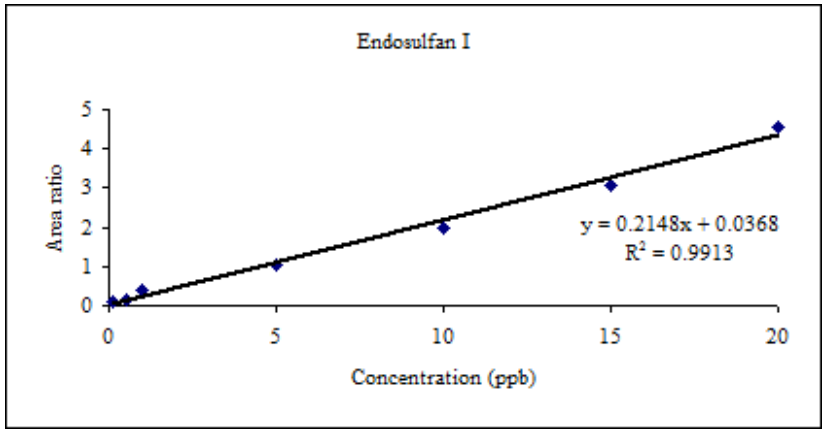
Sampling point	Average area ratio		
	27/05/05	12/10/05	8/12/05
1	0.133116	0.288276	0.624260
2	0.190004	0.112952	0.252716
3	0.133488	0.132987	0.106316
4	0.109639	0.074565	0.241796
5	0.261716	0.255076	0.195396
6	0.252420	0.260388	0.162116
7	0.247108	0.321476	0.314836
8	0.240468	0.301954	0.252420
9	0.245780	0.226112	0.171412
10	0.240473	0.276651	0.415764

2.4 Malathion



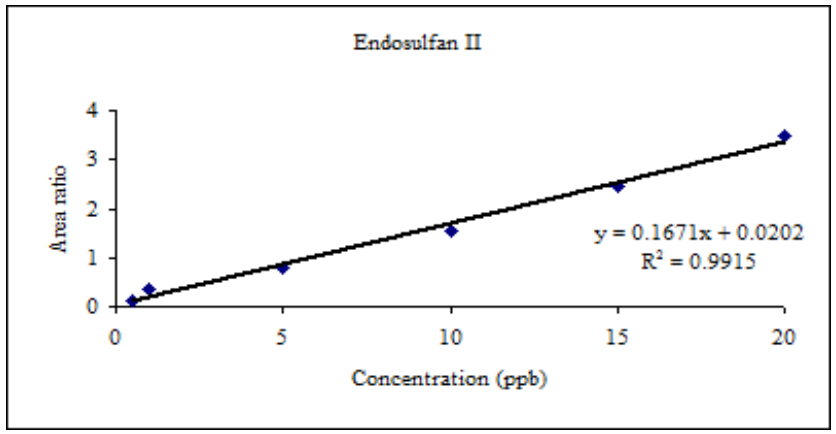
Sampling point	Average area ratio		
	27/05/05	12/10/05	8/12/05
1	0.005892	0.009558	0.007369
2	0.003570	0.022636	0.018213
3	0.045760	0.052496	0.035478
4	0.608726	0.036297	0.006640
5	0.076394	0.050741	0.013642
6	0.016080	0.052338	0.058586
7	0.040863	0.039613	0.048304
8	0.054715	0.003198	0.161760
9	0.027063	0.005151	0.321830
10	0.031457	0.018245	0.156106

2.5 Endosulfan I



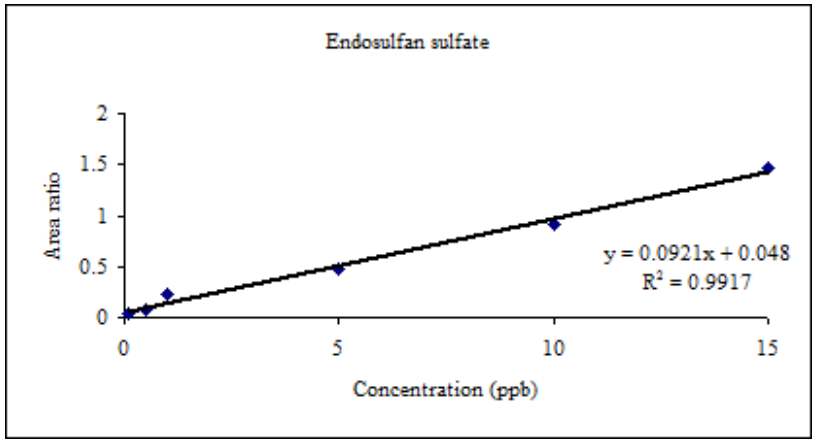
Sampling point	Average area ratio		
	27/05/05	12/10/05	8/12/05
1	0.037294	0.301240	0.036043
2	0.230120	0.258044	0.139904
3	0.165680	0.439116	0.539673
4	0.037638	0.156752	1.553288
5	0.378332	0.146348	0.236564
6	0.318188	0.235198	0.505100
7	0.237132	0.098963	0.065639
8	0.176954	0.212936	0.212936
9	0.284825	0.406342	0.072077
10	0.268784	0.405053	0.303152

2.6 Endosulfan II



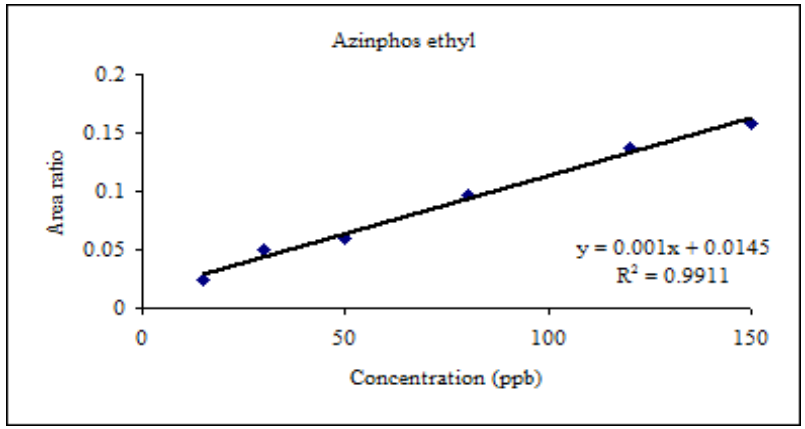
Sampling point	Average area ratio		
	27/05/05	12/10/05	8/12/05
1	0.020969	0.093724	0.020307
2	0.020470	0.020776	0.095395
3	0.077014	0.021023	0.020750
4	0.020306	0.060304	0.571680
5	0.021071	0.020303	0.041900
6	0.262852	0.274193	0.483100
7	0.134184	0.088700	0.061970
8	0.284218	0.115500	0.145525
9	0.020641	0.247460	1.124740
10	0.301284	0.327630	0.115500

2.7 Endosulfan Sulfate



Sampling point	Average area ratio		
	27/05/05	12/10/05	8/12/05
1	0.048421	0.048300	0.048556
2	0.045639	0.048773	0.125400
3	0.061815	0.046550	0.048123
4	0.048296	0.073788	0.211017
5	0.047656	0.047665	0.046567
6	0.089430	0.047043	0.048721
7	0.119832	0.048567	0.049000
8	0.076550	0.048335	0.095892
9	0.048106	0.048970	0.532449
10	0.084840	0.144705	0.059000

2.8 Azinphos Ethyl



Sampling point	Average area ratio		
	27/05/05	12/10/05	8/12/05
1	0.018423	0.015500	0.016528
2	0.014517	0.018340	0.018514
3	0.016633	0.014746	0.016964
4	0.014980	0.016657	0.014501
5	0.016600	0.016886	0.014600
6	0.015483	0.016380	**0.094110**
7	0.014505	0.018877	0.018223
8	0.017376	0.015545	0.014500
9	0.145362	0.014800	**0.070452**
10	0.015336	0.017389	**0.078531**