

**DISTRIBUTION AND ACCUMULATION OF TRIBUTYL TIN (TBT)
IN MARINE ENVIRONMENTS**

The seal of Mahidol University is a large, circular emblem in the background. It features a central golden crown-like symbol (Chhatra) surrounded by a blue ring. The outermost ring contains the university's name in Thai script: "มหาวิทยาลัยมหิดล" (Mahavithayalai Mahidol) at the top and "จุฬาลงกรณ์มหาวิทยาลัย" (Chulalongkornrajavidyalaya) at the bottom.

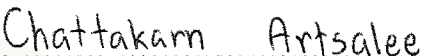
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THE DEGREE OF MASTER OF ENGINEERING
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FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2011**

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Thesis
entitled
**DISTRIBUTION AND ACCUMULATION OF TRIBUTYL TIN (TBT)
IN MARINE ENVIRONMENTS**


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DISTRIBUTION AND ACCUMULATION OF TRIBUTYLtin (TBT) IN MARINE ENVIRONMENTS

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ABSTRACT

The determination of tributyltin (TBT) in four shellfish species, such as *Paphia undulata*, *Solen regularis*, *Anadara granosa*, and *Perna viridis*, including marine water, and sediment collected from aquaculture areas of each species, and the study of the interaction among these various kinds of samples were carried out under this research. Validation was performed to ensure the methods are suitable for all samples, extraction efficiency and percent recovery of TBT analytical methods for all mediums were more than 90 %. TBT concentrations in marine water, shellfish, and sediment ranged from 0.90-1.30 ng ml⁻¹, 40.92-119.10 ng g⁻¹ dry weight, and 104.34-212.53 ng g⁻¹ dry weight, respectively. Considering to TBT physico-chemical properties, when introduced into water it will partly adsorb with particulate matter. Microorganisms and UV light are the important factors for degradation of TBT once it is adsorbed. TBT degradation rates in sediment are slower than in the water column. Therefore, the highest TBT concentration was found in sediment samples while the lowest TBT concentration was found in marine water. In addition, TBT contaminations in shellfish, marine water, and sediment have correlation with each other. The highest TBT contamination in three mediums was found in the mariculture site of *Paphia undulata*. Sources of TBT might come from high boating activities along Mae Klong River and wastewater discharged from the industries. *Paphia undulata* lives on the sea floor where photolysis might be inhibited and biodegradation of TBT slowly takes place in sediment. Such benthos might absorb TBT while they feed. The concentrations of TBT in shellfish under this study were assessed for the potential risk to human health. Daily intake of shellfish consumption in those shellfish species should not be more than 0.86 kg for *Anadara granosa*, 1.33 kg for *Solen regularis*, 1.40 kg for *Paphia undulata*, and 2.51 kg for *Perna viridis*. The daily consumption of shellfish was inversely proportional to the level of TBT in those shellfish species. Based on the lowest TBT concentration in shellfish under this study of 2.37 ng g⁻¹ wet weight, daily shellfish consumption should not be more than 6.33 kg.

KEY WORDS: TRIBUTYLtin / MARINE WATER / SEDIMENT / PHOTOLYSIS / BIODEGRADATION

96 pages

การกระจายตัวและการสะสมของไตรบิวทิลทินในสิ่งแวดล้อมทางทะเล

DISTRIBUTION AND ACCUMULATION OF TRIBUTYLTIN (TBT) IN MARINE ENVIRONMENTS

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บทคัดย่อ

งานวิจัยนี้ถูกจัดทำขึ้นเพื่อศึกษาการสะสมของ TBT ในตัวอย่างหอย *Paphia undulata*, *Solen regularis*, *Anadara granosa*, และ *Perna viridis*, รวมถึงน้ำทะเล และดินตะกอนจากแหล่งเพาะเลี้ยงตามธรรมชาติ การตรวจสอบความใช้ได้ของวิธีทดสอบและร้อยละการกลับคืนมีค่าสูงถึง 90 % ความเข้มข้นของ TBT ในตัวอย่างน้ำทะเล หอย และดินตะกอนมีค่า 0.90-1.30 ng ml⁻¹, 40.92-119.10 ng g⁻¹ (น้ำหนักแห้ง) และ 104.34-212.53 ng g⁻¹ (น้ำหนักแห้ง) ตามลำดับ จากผลการทดลองพบว่า TBT มีการสะสมในน้ำทะเลน้อยที่สุด ทั้งนี้เนื่องจากการย่อยสลายของ TBT จากจุลินทรีย์และแสงอาทิตย์ ในขณะที่ TBT ในดินตะกอนมีความเข้มข้นสูงสุด เนื่องจากการย่อยสลายของจุลินทรีย์เกิดขึ้นได้น้อย การสะสมของ TBT สูงสุดในตัวอย่างน้ำทะเล หอย และดินตะกอนพบในแหล่งเพาะเลี้ยงหอย *Paphia undulata* ซึ่งได้รับผลกระทบจากการหลุดลอกของ TBT จากเรือประมง และการลักลอบปล่อยน้ำเสียจากโรงงานอุตสาหกรรมบริเวณแม่น้ำแม่กลอง นอกจากนี้ยังพบว่าการสะสมของ TBT ในตัวอย่างทั้งสามชนิดมีความสัมพันธ์กัน เนื่องจาก *Paphia undulata* อาศัยอยู่ในโคลนน้ำลึกที่ซึ่งการย่อยสลายทางชีวภาพเกิดขึ้นอย่างช้าๆ จึงทำให้หอยหน้าดินชนิดนี้ได้รับผลกระทบจาก TBT ซึ่งสะสมอยู่ในดินตะกอนด้วยเช่นกัน จากผลการวิเคราะห์ปริมาณ TBT ในตัวอย่างหอยนำไปสู่การคำนวณหาปริมาณสูงสุดของการบริโภคหอย *Anadara granosa*, *Solen regularis*, *Paphia undulata*, และ *Perna viridis* ทั้งสี่ชนิดที่ไม่เป็นอันตรายต่อสุขภาพของผู้บริโภค ดังนี้ 0.86 kg, 1.33 kg, 1.40 kg, และ 2.51 kg ต่อวัน ตามลำดับ และเนื่องจากปริมาณการบริโภคหอยแปรผกผันกับปริมาณ TBT ที่สะสมอยู่ในเนื้อหอย ความเข้มข้นต่ำสุดของ TBT ในตัวอย่างหอยซึ่งวิเคราะห์ได้จากงานวิจัยนี้มีค่า 2.37 ng g⁻¹ (น้ำหนักเปียก) ดังนั้นปริมาณการบริโภคหอยสูงสุดจึงไม่ควรเกิน 6.33 kg ต่อวัน

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CHAPTER I

INTRODUCTION

1.1 Introduction

Nowadays, a variety of environmental problems affect our entire world. The contamination of pollutants is likely to have impacts on environmental quality or harmful to health and hygiene of the public. Refuse, polluted air, wastewater or any other hazardous substances which are discharged from point sources of pollution, are the cause of soil pollution, air pollution, and water pollution. Moreover, polluted runoff from agriculture areas draining into a river, or wind-borne debris blowing out to sea are nonpoint source pollution which cause water and air pollution. As globalization continues and the earth's natural processes transform local problems into international issues.

About 71% of the earth surface is covered with marine water, the remainder consisting of continents and islands; liquid water, necessary for all creature. Wastewater from the industries, leachate from landfills, and the contamination of toxic chemical in the water bodies are the major sources of water pollution. When the water is polluted, the aquatic animals and plants cannot survive. Hazardous chemicals disperse rapidly in the sea but some toxicity collected in the sediment and affect to benthic animals.

Organotin compounds are also hazardous chemicals and have been used since the 1940s in plastics industries as polyvinyl chloride (PVC) stabilizers and as catalysts for polyurethane foams. Once their biocide properties were discovered in 1950s, they were used as the active compound in antifouling paints applied on ship hulls and harbor facilities to prevent settling of barnacles, seaweeds or tubeworms (Hoch, 2001). The release of triorganotin compounds from immersed structures is one of the main problems of diffuse and continuous contamination of the marine environment.

Many researches have shown that triorganotins effect on non-target marine organisms, i.e. mortality of the larvae of mussels, shell malformation in oysters, and the development of male sexual characters form in female gastropods (imposex) (WHO, 1990). However, the effects on humans have not yet been studied in detail. Thus, understanding the fate of tributyltin (TBT) in the environment is therefore of primary importance to prevent its migration.

1.2 Statement of The Problems

Organotins are widely used as pesticides, additives in antifouling paints, preservatives in wood, textile, leather and paper, stabilizers in the manufacture of PVC, and for cooling pipes of thermoelectric power. Organotin antifouling paints were introduced as the boat-bottom paints in the early 1960s to prevent settling of barnacles, seaweeds or tubeworms (Hoch, 2001). Almost all vessels in the world used these antifouling paints until the mid 1980s that the researchers from France and the UK began to suggest that the use of TBT in the antifouling paints was adversely impacting a number of marine species other than the fouling organisms (Omae, 2006).

Toxic effects of organotin compounds on aquatic organisms and mammals are well known and also have great concern on humans. The leaching rates of organotin compounds from the antifouling paint film into seawater from one vessel are very low. However, as too many vessels release organotin compounds into seawater, the amount of organotin compounds in seawater in closed sea areas such as harbors and marines, gradually increases year by year (Omae, 2006).

Many studies have shown that TBT is not persistent in seawater. Due to TBT is stable in the sediment, sludge and living organisms, moreover, the particular concern is the stability of TBT in contaminated sediment where biodegradation process may be inhibited (Omae, 2006). In addition, the contamination of TBT in sediments is mostly reported high concentrations at coastal areas especially where high harbor activities occur (Seneewongse Na Ayudthaya, 2006).

The “International Convention on the Control of Harmful Antifouling Systems on Ships” adopted on 5 October 2001 by the International Maritime Organization (IMO) stated that: (1) by 1 January 2003, a ban on the application of TBT-based antifouling paints should be introduced; and (2) 1 January 2008 is the last

date for having a TBT-based antifouling paint on a vessel (Omae, 2006). Although the control was approved in 1999 by the IMO conference, by November 2005 it had still not come into effect.

An increase of human and industrial activities has imposed considerable contamination by toxic chemicals effect on aquatic environment (Hungspreugs et al., 1989). Thailand is one of developing countries in Southeast Asia which has the high rising rate of manufacturing activities. However, there have been a few researches conducted by government organizations whose work is directly involved with environmental contamination (Doungkaew, 2006).

There are some studies which reported the contamination of butyltin compounds in the Asia-Pacific region. Tanabe, *et al.* (2000) examined the contamination of butyltin (BTs) and organochlorine compounds (OCs) in green mussels collected along the coastal areas of Thailand, Philippines and India during the period of 1994 to 1997. Bech (2002) investigated how tributyltin contamination resulting from the establishment of a new marina and increasing yachting activities at Phuket Island, Thailand.

According to a few researches working on the TBT associated with organisms in Thailand. It is worthy of investigation for scientific reason, therefore, the validation of TBT analytical methods for marine biota, marine water, and marine sediment was performed in order to guarantee the methods are suitable for the real samples. Consequently, the concentrations of TBT contamination in marine biota, marine water, and marine sediment collected from the mariculture areas were determined. TBT levels in shellfish, marine water, and sediment were used to study the correlation of TBT among those mediums. In addition, the results of TBT concentration in shellfish were used to estimate the possible risk to human health resulting from seafood consumption.

1.3 Research Objectives

This research was held on these following objectives:

- 1.3.1 To validate analytical methods for TBT analysis with gas chromatograph-mass spectrometer (GC-MS).
- 1.3.2 To determine the concentrations of TBT in shellfish, marine water, and sediments collected from Thailand coastal mariculture.

- 1.3.3 To investigate TBT contamination in shellfish which effects on human health as well as food web.

1.4 Scope of Study

To accomplish the above objectives following tasks are covered in the scope of this study:

- 1.4.1 The optimum condition for TBT analysis in marine water, sediments, and biotas was validated in order to confirm the analytical procedures appropriate for the real samples.
- 1.4.2 Razor clam (*Solen regularis*), undulated surf clam (*Paphia undulata*), blood cockle (*Anadara granosa*), and green mussel (*Perna viridis*) were collected from Maha Chai Market and Don Hoi Lot Market for the analysis in order to determine the concentration of TBT by using GC-MS.
- 1.4.3 Shellfish, marine water, and sediment were collected from four different aquaculture areas of species and the monitoring of TBT concentration was performed by using GC-MS.
- 1.4.4 The results of TBT concentration in shellfish species were used to evaluate the risk on human health.

CHAPTER II

LITERATURE REVIEW

2.1 Tributyltin Compounds

2.1.1 Tributyltin Properties

The tributyltin (TBT) compounds are organic derivatives of tetravalent tin. Organotin compounds are classified as R_4Sn , R_3SnX , R_2SnX_2 , and $RSnX_3$. In compounds of industrial importance, R is usually a butyl, octyl, or phenyl group and X is a chloride, fluoride, oxide, hydroxide, and carboxylate (WHO, 1980). TBT compounds exist as seven species, oxide, benzoate, chloride, fluoride, linoleate, methacrylate, and naphthenate (Table 2.1).

Tributyltin has a low aqueous solubility and relatively high affinity for particulate matter, providing a direct and potentially persistent route of its entry into benthic sediment (Omae, 2006). The solubility in water is between <1.0 and >100 mg/L according to pH, temperature, and anions present in the water. In sea water and under normal conditions, TBT exists as three species (hydroxide, chloride, and carbonate), which remain in equilibrium. At pH values less than 7, the predominate forms are $Bu_3SnOH_2^+$ and Bu_3SnCl , at pH 8, they are Bu_3SnCl , Bu_3SnOH , and $Bu_3SnCO_3^-$, and at pH values above 10, Bu_3SnOH and $Bu_3SnCO_3^-$ predominate (WHO, 1990).

The initial test showed the result of the sorption of TBT to the sediment was taken up within 10 min. and the equilibrium was achieved within 2 hours, when 85.7% of the added TBT had bound with suspended particles in the dark at 15°C (Figure 2.1). The difference of affinities of the organotin compounds with water and sediment relates to the difference of the half-lives of organotin compounds in water and the sediment (Omae, 2006).

2.1.2 Occurrence and Distribution of Tributyltin

In the 1950s, Dutch scientists first recognized the biocidal properties of triorganotin compounds. It was found that the different triorganotin compounds have

Table 2.1 Identity and physical and chemical properties of tributyltin compounds (WHO, 1990)

	Oxide (TBTO)	Benzoate (TBTB)	Chloride (TBTCI)	Fluoride (TBTF)	Linoleate (TBTL)	Methacrylate (TBTM)	Naphthenate (TBTN)
IUPAC name	distannoxane, hexabutyl	stannane, (benzyloxy) tributyl	stannane, tributyl-chloro	stannane, tributyl-fluoro	stannane, tributyl- (1-oxo-9,12-octa decadienyl)oxy-	stannane, tributyl- (2-methyl-1-oxo-2- propyl)oxy-	stannane, tributyl- mono (naphthenoy loxy) derivatives
CAS name	Bis(tributyltin) oxide	Tributyltin benzoate	Tributyltin chloride	Tributyltin fluoride	Tributyltin linoleate	Tributyltin methacrylate	Tributyltin naphthenate
CAS number	56-35-9	4342-36-3	1461-22-9	1983-10-4	24124-25-2	2155-70-6	85409-17-2
Molecular formula	$C_{24}H_{54}OSn_2$	$C_{19}H_{32}O_2Sn$	$C_{12}H_{27}ClSn$	$C_{12}H_{27}FSn$	$C_{30}H_{58}O_2Sn$	$C_{16}H_{32}O_2Sn$	
Relative molecular mass	596	411	325	309	568.7	374.7	~500
Boiling point (°C)	173 (130 Pa)	~135 (30 Pa)	140 (1300 Pa)	>350 (extrapol)	~140 (50 Pa)	>300 (extrapol)	~125 (50 Pa)
Melting point (°C)	< -45	20	-16	240	< 0	16	< 0
Relative density (20°C)	1.17-1.18	~1.2	~1.2	1.25	1.05	1.14	~1.1
Vapor pressure (Pa at 20°C)	1×10^{-3}	2×10^{-4}			9×10^{-2}	3×10^{-2}	9×10^{-5}
Refractive index (20°C)	1.4880-1.4895						

different toxicities to different organisms. Tributyltin was found as the most toxic compound of triorganotins to gram-positive bacteria and to fungi. For a decade after, in the early 1960s, the first use of tributyltin oxide (TBTO) and tributyltin fluoride were occurred in Africa as molluscicides against the freshwater snails which are vectors of the disease schistosomiasis, the snails being the intermediate hosts of the trematode parasite (WHO, 1990).

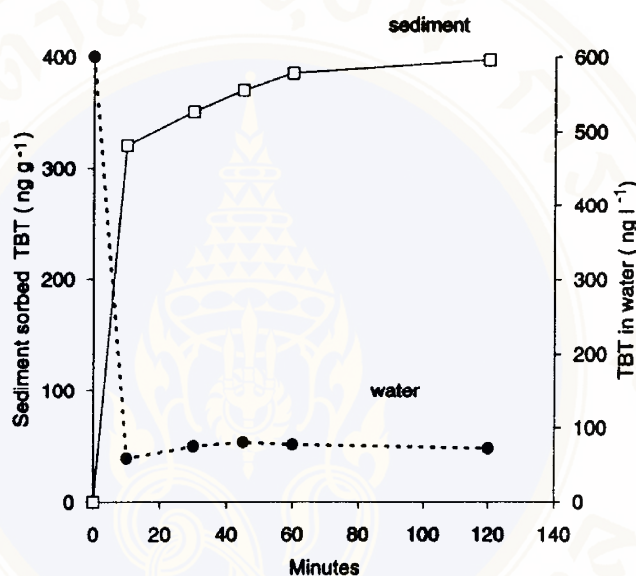


Figure 2.1 The equilibration of TBT between particulates and water. The vessel initially containing 500 ml water, 1 g sediment and 300 ng ¹⁴C-labeled TBT (Omae, 2006)

Organotin compounds are used in a wide range of everyday products and in a several of technical applications. The worldwide production of organotin compounds has risen from the lowest level in the late 1940s to 5,000 tonnes in 1955, to 25,000 tonnes in 1975, and to about 50,000 tonnes per year in 1990 (Visoottiviseth, 2001). Nowadays, organotin compounds are used; (1) as agricultural fungicides (triphenyltin acetate, triphenyltin hydroxide); (2) as general biocides (bis(tributyltin) oxide) in paints and in preservation of leather, textiles, paper, and wood; (3) as disinfectants for use in hospitals (tributyltin benzoate); (4) as helminthicides in poultry (dibutyltin dilaurate, tetraisobutyltin); (5) as nematocides (trivinyltin chloride); (6) as rodent repellents (tributyltin chloride, triphenyltin chloride and acetate); (7) as molluscicides (triphenyl- and tributyltin compounds); and (8) as antifoulants in ship

paints and underwater coating (triphenyl- and tributyltin compounds) (WHO, 1990). A schematic diagram of the application of organotin compounds is shown in Figure 2.2.

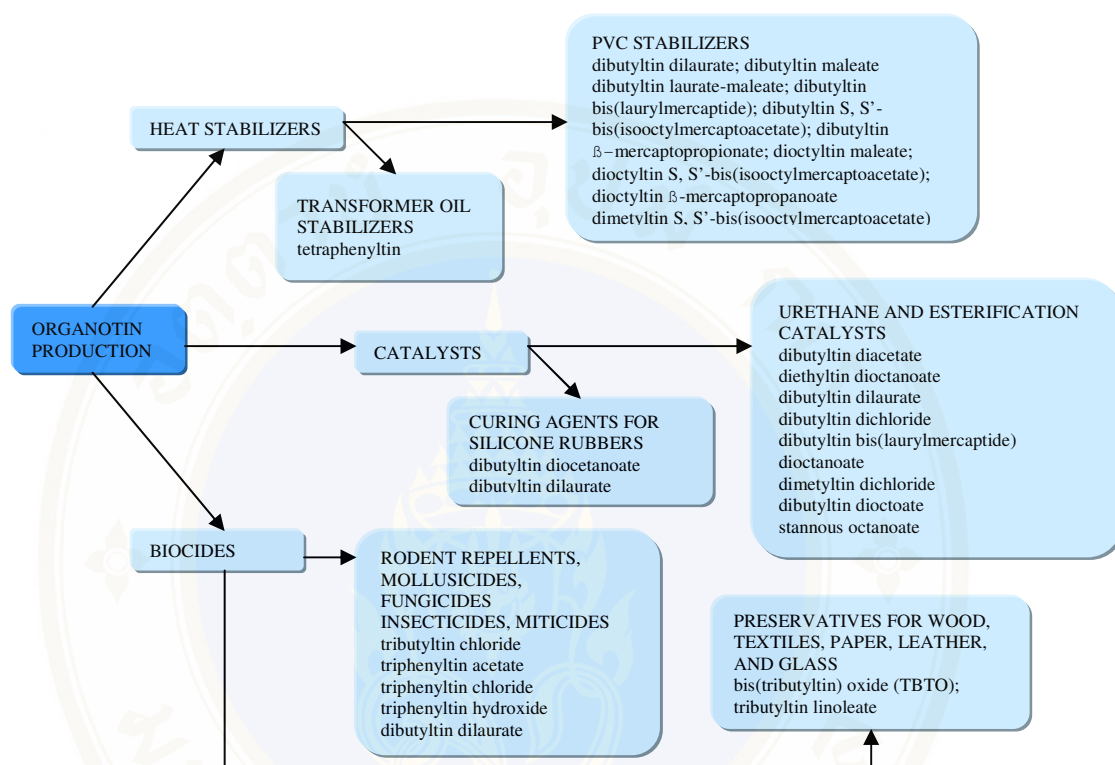


Figure 2.2 A schematic diagram of the various uses of organotin compounds (WHO, 1980)

The 4 categories of organotin compounds, dibutyltin derivatives (e.g. dibutyltin dilaurate, dibutyltin diacetate) are the most important commercially. In addition, dialkyltin derivatives were used to protect plastic product from degradation during use, as vulcanizing agents for silicone rubbers, and as catalysts in the production of polyurethane foam products. Trialkyltin derivatives were used in agriculture as non-systemic fungicides and acaricides. Tetraalkyltin derivatives were used as intermediates in other organotin compounds manufacture. Monalkyltin derivatives have a limited commercial use as heat stabilizers for PVC plastic films.

The use of organotin compounds depends on 2 features: nonbiocidal and biocidal. Nonbiocidal, since 70% of the total production of organotin compounds is used to heat-stabilize poly (vinyl chloride) plastic products to prevent degradation of the polymer during the melting and forming of the resin into its final products.

The biocidal applications are about 30% of the total use. The compounds involved mainly triorganotin (Table 2.2).

Table 2.2 Biocidal uses of triorganotin compounds (Visoottiviseth, 2001)

Agrochemical Fungicides	Ph ₃ SnX
Agrochemical Miticides	Cy ₃ SnX and Np ₃ SnX
Wood Preservative Fungicides	Bu ₃ SnX
Marine Antifouling Paint Biocides	Bu ₃ SnX and Ph ₃ SnX
Disinfectants	Bu ₃ SnX
Stone Preservatives	Bu ₃ SnX
In-Can Paint Biocides	Bu ₃ SnX

2.1.3 Degradation of Organotin Compounds

Once tributyltin was introduced into natural waters will partly adsorb onto particles due to its physico-chemical properties. The adsorption ability depends on conditions, such as salinity, concentration, and organic content of particulate matter. When it is adsorbed, decrease in TBT concentration takes place mainly by degradation. It is known that TBT degradation rates in sediment are slower than in the water. The degradation of the organotin compound may be defined as the progressive removal of the organic groups from tin atom. It is theoretically completed with the liberation into water of the tin oxide (SnO₂) (WHO, 1990).



When R = organic groups, and X = anionic groups

The results of laboratory studies have shown that triorganotin compounds experience degradation in the environment via progressively less toxic di-organotin and mono-organotin derivatives, to form a harmless inorganic tin residue (Omae, 2006). Degradation associates the breaking of the tin-carbon bonds and this could occur by different processes; UV irradiation, biological cleavage, and chemical cleavage.

The tin-carbon bond in the TBT structure is a strong bond which is stable in the presence of water, atmospheric oxygen and temperature up to 200°C. Therefore, there is no significance of thermal decomposition under environment conditions (Doungkaew, 2006). The degradation of the organotin compounds possibly occur in both abiotic degradation (hydrolytic cleavage of the tin-carbon bond and photodegradation) and biotic degradation. Hydrolytic cleavage occurs only under conditions of extreme pH. It is hardly evident under normal environmental conditions (WHO, 1990).

Photodegradation of TBT by UV light is theoretically possible. UV light contains wavelengths in the range of 10-400 nm, wavelengths of UV light shorter than 290 nm is absorbed by a thin layer of ozone in the atmosphere. However, UV light with a wavelength longer than 290 nm possesses an energy of approximately 300 kJ/mol, whereas the energy required to break the tin-carbon bond is 190-220 kJ/mol. Then, it is impossible to break tin-carbon bond by UV irradiation. Moreover, the photodegradation of TBT yielded to the derivatives of dibutyltin seemed to be resistant to photolysis because little amount of monobutyltin was formed (WHO, 1990).

The most dominant degradation is caused by microorganisms such as bacteria, algae and fungi (Omae, 2006). According to WHO (1990), biodegradation kinetics depend on environmental conditions, such as temperature, oxygenation, pH, the level of mineral elements, presence of biodegradation organic substances, and the nature and adaptation of micro flora. A schematic diagram of environmental degradation involving microorganisms for tributyltin and triphenyltin (TPT) compounds is given in Figure 2.3.

A mechanism of biodegradation also exists under anaerobic conditions (Maguire and Tkacz, 1985). Aerobic degradation of ^{14}C -labeled TBTO in soil is considered to be faster than anaerobic conditions (Omae, 2006). ^{14}C -labeled triphenyltin acetate, bis(tributyltin) oxide, tributyltin fluoride, triphenyltin chloride, and triphenyltin fluoride in soil could be broken down to inorganic tin. Since carbon dioxide was evolved, and a similar breakdown did not occur in sterile soil, the degradation was ascribed to the ability of certain microorganisms to metabolize the organotin compounds (Barnes *et al.*, 1973).

A number of species were found to be capable of conducting such degradation aerobically; gram-negative bacteria (*Pseudomonas aeruginosa* and

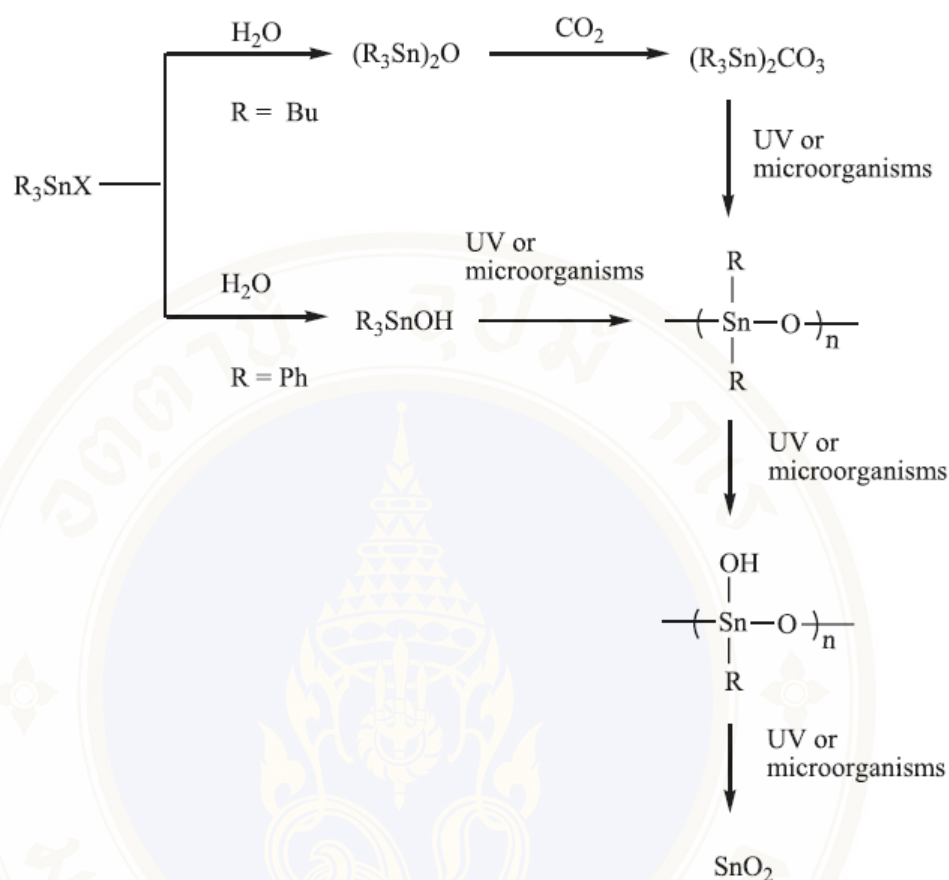


Figure 2.3 Environmental degradation scheme for TBT and TPT compounds (Omae, 2006)

Alcaligenes faecalis), wood-degrading fungi (*Coniophora puteana*, *Trametes versicolor*, and *Chaetomium globosum*), and the green alga (*Ankistrodesmus falcatus*) are reported as dealkylation organisms (Barug and J.W., 1980). Temperature is one of the important factor which influences rate of degradation. A colder temperature slowed the degradation of TBT takes place by microorganisms in the marine environment as shown in Table 2.3.

Since triorganotin compounds were used as an active ingredient of antifouling paints, the release of TBT from immersed structures protected by organotin based antifouling paints has been responsible for the problems of the direct, diffuse and continuous contamination of the marine environment. These organotins are degraded by hydrolysis in water with sunlight and microorganisms at a room temperature. Therefore, data for the half-lives in natural seawater, in fresh water or in pure water in the laboratory vary with the intensity of light, temperature, types or

amounts of organisms, flow rates of seawater, types or amounts of ions in seawater, and amounts of the organotin compounds (Omae, 2006).

Table 2.3 Degradation of TBT in the marine environment (Omae, 2006)

Comments	Initial TBT concentration (µg/L)	Reaction order	Half-life	Rate constant
Natural water				
-Radiolabeled TBT	0.615	First	3.5 days	0.20/day
-Ambient marine water	0.57		6 days	
-Natural water	0.8	First	60 days (at 5°C)	
-Filtered seawater	0.55	First	2.5 days	0.28/day
Sediment				
-Sediment cores from a marine	0.3	First	1.85 years	0.375/year
Biota				
-Eelgrass grown in TBT-spiked seawater	0.14	First	9.6 days	0.072/day

Among aquatic organisms, molluscs and fishes have very high TBT accumulations. Short and Thrower (1986), studied bioaccumulation in salmon (*Oncorhynchus tshawytscha*) exposed for 96 hours to concentrations of 1.49 µg/L. Tsuda, *et al.* (1987) investigated the accumulation of TBTO in carp (*Cyprinus carpio*) exposed for 14 days to concentrations varying between 1.8 and 2.4 ng/L. Cheng and Jensen (1989) determined tin uptake and water concentrations of tin over a period of 51 days by transfer mussel (*Mytilus edulis*) from an unpolluted area into net bags suspended in a marina in Denmark. A study showed that the depurations of TBT from gill tissue and digestive gland tissue involves a rapid TBT, half-life values range from 2.2-5.3 days, whereas 28-69 days for a slow TBT as shown in Table 2.4.

Table 2.4 Half-life values for the depuration of TBT and DBT in mussels (Omae, 2006)

Tissue	TBT rapid $T_{1/2}$ (day)	TBT slow $T_{1/2}$ (day)	DBT $T_{1/2}$ (day)
Whole animal	-	69	115
Gonad	-	28	58
Digestive gland	5.3	58	35
Gills	2.2	53	36

Tributyltin was found to degrade rapidly to dibutyltin (DBT) in a marina where high TBT concentrations have been reported; their half-lives are about several days to several months (Table 2.5). Most of TBT was lost from the water column through biodegradation, two-thirds was degraded through debutylation to DBT. One-third of the TBT was degraded directly to monobutyltin (MBT) in the water. Another portion of the TBT removed from the water column was transported rapidly to the air-water interface to a measurable degree.

Table 2.5 Half-life values of TBT in water (Omae, 2006)

Organotin, medium	Half-life (days)	Comment (initial concentration)	Publication year
TBT, seawater	4-19		1989
TBT, seawater	6-7	(0.5 µg/L)	1989
TBT, seawater	17	open seawater (28°C)	1986
TBT, seawater	6-13	in sunlight, San Diego, USA	1996
TBT, seawater	7-19	in the dark, San Diego, USA	1986
TBT, seawater	6	in sunlight, San Diego, USA	1988
TBT, seawater	13	in one location, no degradation over 15 days in the dark, Osaka, Japan	1988
TBT, seawater	4-19	in sunlight, USA	1989
TBT, seawater	15	Italy	1990
TBT, seawater	6	in the light, ¹⁴ C TBT (2 µg/L)	
	7	in the dark, USA, marina	1988
TBT, seawater	17	open seawater	1996
TBT, seawater	60	at 5°C	1987
TBT, estuarine	6-7	at 28°C	1996
TBT, estuarine	7-14	at 22-28°C in the dark	1988
TBT, estuarine	7.77	0-39 days	1998
	2.55	for first 10 days	
	13.4	after 11 to 40 days	
TBTO, fresh water	>89	in sunlight	1983
TBT, fresh water	26	in winter, in lab window	1992

The contamination of the organotin compounds in sediment have been reported to be about 1 to 15 years. Degradation of TBT in sediment has been studied with various half lives. Removal of tributyltin from contaminated sediment is caused by both degradation in sediment and release to the overlaying water. In particular, TBT is stable in sediment, sediment may be a big reservoir for TBT in some locations. The half-life values of organotin compounds are shown in Tables 2.6.

Table 2.6 Half-life values of TBT in sediments (Omae, 2006)

Organotin, medium	Half-life	Comment (initial concentration)	Publication year
TBT, sediment	about 2.5 years	Auckland, New Zealand	1995
TBT, sediment	about 8.7 years	Vancouver, Canada	1997
TBT, sediment	2.1 years		1995
TBT, sediment	1.85 years, first order 0.375/year, estimated from sediment core data	Auckland, New Zealand (TBT: 0.759 $\mu\text{gSn/g}$)	1989
TBT, sediment	>8-15 years, estimated from sediment core data	Arcachon Bay, France	1990
TBT, sediment	6.9 years, estimated from sediment core data	Chinhae Bay, Korea	1998
TBT, sediment	2.1 years, estimated from sediment core data	Oleron Island, France	1995
TBT, sediment	100-800 days, faster under aerobic conditions than anaerobic conditions	Japan	1995
TBT, sediment	460 days anaerobic sediment	Toba Bay, Japan	1992
TBT, sediment	360-775 days surficial sediment	UK	1993

2.1.4 Bioaccumulation of Triorganotin Compounds

The lipophilic properties of TBT and its moderately high octanol-water partition coefficient ($\log P_{ow} > 3$) contribute to bioaccumulation in living organisms (WHO, 1990). Many laboratory experiments have been operated under different conditions to demonstrate the accumulation of TBT in marine sediment and marine biota. The results implied that degradation processes operating in the sediment and biota will be of greater importance in determining the overall persistence of TBT in the marine environment.

The concentrations of TBT in seawater, sediment, and marine biota are shown in Figure 2.4. These data show that TBT is stable in the sediment, sludge and living organisms such as algae, mussels and fish (Omae, 2006). According to WHO (1990), TBT levels have been found to reach $1.58 \mu\text{g/L}$ in marine water and estuaries, $7.1 \mu\text{g/L}$ in fresh water, $26,300 \mu\text{g/kg}$ in coastal sediments, $3700 \mu\text{g/kg}$ in fresh water sediments, 6.39 mg/kg in bivalves, 1.92 mg/kg in gastropods, and 11 mg/kg in fish.

The high contamination of TBT in sediments was found especially at coastal areas, however, far away from coastal region, there are DBT detection in the groundwater and freshwater (Hoch, 2001). In principle, contaminants can reach the environment both directly, via air or water or soil from the production processes, use, and disposal and indirectly as a consequence of biotic and abiotic transformations. Figure 2.5 shows the distribution and fate of organotin compounds and their general routes into marine environment.

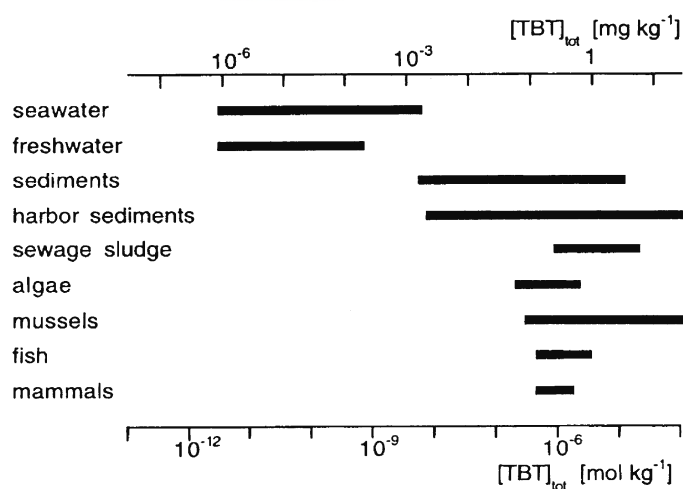


Figure 2.4 TBT concentration in different compartments of marine environments (Omae, 2006)

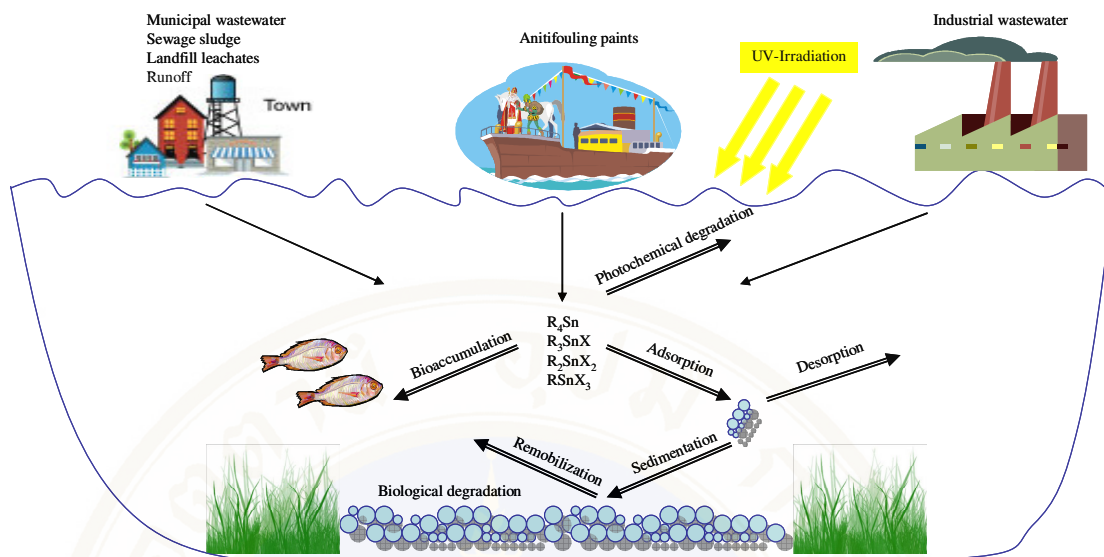


Figure 2.5 The distribution of organotin compounds in the aquatic environment (Hoch, 2001)

2.1.5 Toxicity of Triorganotin Compounds

Inorganic tin is considered as being non toxic because the metal does not react and the oxides are insoluble at physiological pH. However, the organic series, R_nSnX_{4-n} (R =carbon-bonded organic group; X =inorganic group; and $n=1-4$), with trialkyltin ($n=3$) compounds being the maximum toxicity to all types of living organisms. Both forms of tin compounds entering mammals through food are excreted within a short period of time (Sherman, 1986). Tributyltin and triphenyltin are used to control bacteria and fungi without apparent adverse effects to the people who apply them in a short period of time but they are highly toxic to fish and phytoplankton.

TBT is more toxic to gram-positive bacteria (minimal inhibitory concentration (MIC) between 0.2 and 0.8 mg/L) than to gram-negative bacteria (MIC: 3 mg/L). Toxicity to marine microorganisms is difficult to set no-observed-effect level (NOEL) values but lies below 0.1 $\mu\text{g/L}$ for some species. Besides, TBT could cause acute and chronic effects to organisms by having lethal concentrations in the ranges of 0.04-16 $\mu\text{g/L}$ for short-term exposures (WHO, 1990).

According to WHO (1980), the experimental application of organotin compounds (e.g., dibutyltin dichloride and tributyltin chloride) to the skin of volunteers showed that they produced skin irritation in workers 1-8 hours after contact. Di- and tri-butyltin compounds caused eye irritation after brief contact. A 20%

solution of triphenyltin acetate produced irritation of the skin and the mucous membranes of the upper respiratory tract. Toxic effects on fish and aquatic organisms can be divided into two categories; acute and chronic effects.

Acute effects are used to measure the mortality of aquatic organisms and are usually presented in terms of median lethal concentration (LC_{50}) or median effective concentration (EC_{50}). LC_{50} is the concentration of material in water which results in 50% death of the test organisms. EC_{50} is the concentration of material in water which results in reducing some sublethal response in 50% of the organisms when exposed (Visoottiviseth, 2001). According to WHO (1990), the laboratory experiment showed that TBT affects shell deposition of growing oysters, gonad development and gender of adult oysters, settlement, growth, and mortality of larval oysters and of other bivalves.

Chronic effects may take place in a long period of time with very low concentration of organotin compounds which cause disorders, for example, imposex symptom (Figure 2.6) being used to describe the development of male characteristics, such as a penis and vas deferens in female gastropods. This phenomenon was found worldwide in *Nassarius obsoletus* (Smith, 1981), *Nucella lapillus* (Bryan *et al.*, 1986), *Hexaplex trunculus* (Terlizzi *et al.*, 1998), *Cronia margariticola* (Astilla *et al.*, 2005), and *Thais brevidentata* (Gravel *et al.*, 2006).

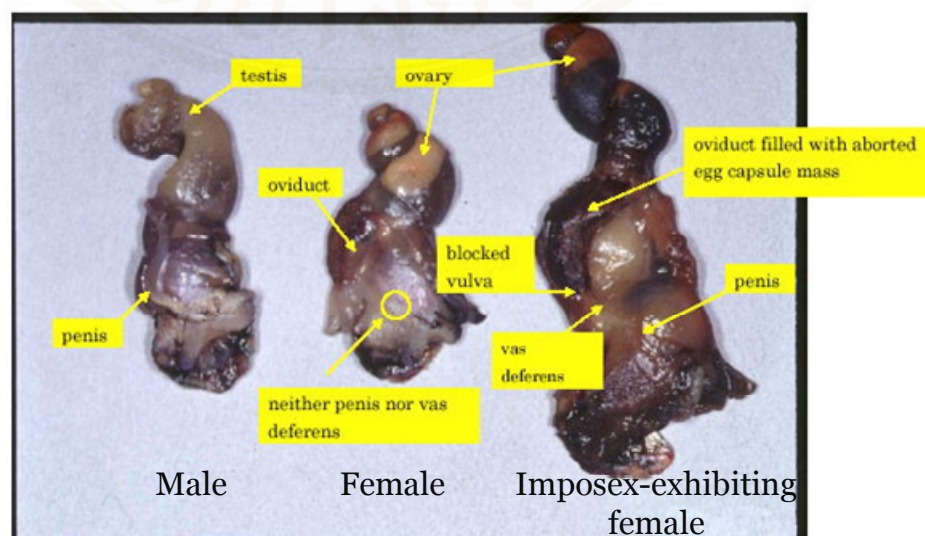


Figure 2.6 Imposex symptom in Rock Shell (*Thais clavigera*)

(National Institute for Environmental Studies, 2002)

Tin is a ubiquitous element. In fact, human bodies contain 0.2 ppm of tin. Food is the main source of exposure to tin for man (Omae, 2006). According to Hoch (2001), the exposure of human to organotin compounds can be accounted to the contamination in food and the household products as shown in Figure 2.7. TBT shows the highest toxicity to non-target aquatic organisms, such as algae, zooplankton, molluscs, and some fishes. Leaching of TBT from paint matrix of ships and its breakdown products, DBT and MBT, are accumulated through aquatic food chain.

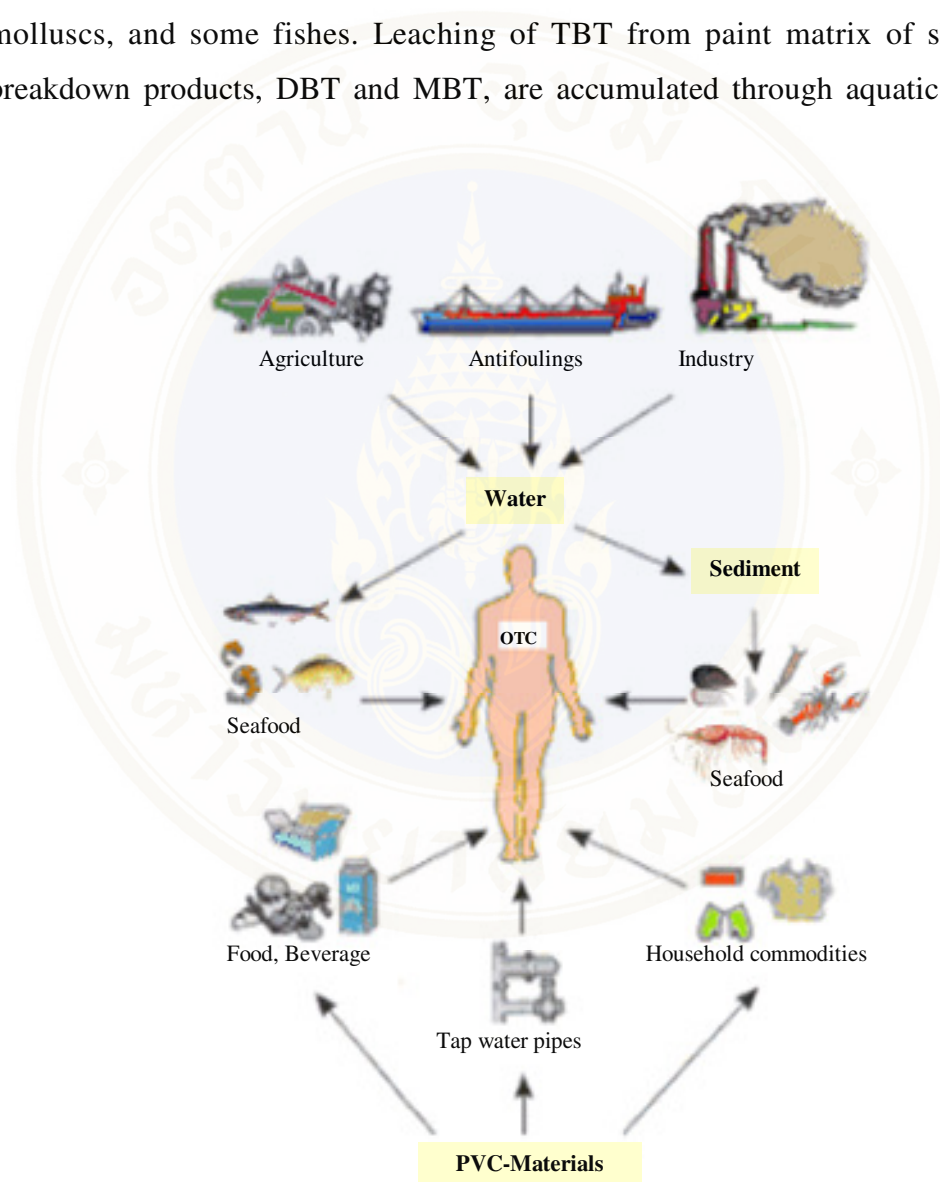


Figure 2.7 General sources of contaminated organotin compounds to human (Hoch, 2001)

The detection of butyltin compounds in livers of human was lower than fish and marine mammals (Kannan and Falandysz, 1997). The concentration of DBT and MBT in human liver samples were found at 9 ng/g and 1.6 ng/g, respectively,

whereas, the concentration of TBT was less than 0.3 ng/g (Nielson and Strand, 2002). Since TBT compounds can produce severe irritation of the skin, mucous membrane, and the upper respiratory tract, the application of them must be prevented. From the available data, there is neither evidence that TBT can damage to the central nervous systems, nor it is the cause of carcinogenic in humans.

2.2 Shell Species

2.2.1 *Solen regularis*

Solen regularis (Figure 2.8) is found in Don Hoi Lot, a rare type of natural wetland in Thailand, comprising sandbars at the mouth of the Mae Klong River with a vast area of tidal mudflats in the Muang District, Samut Songkhram Province. *Solen regularis* live buried vertically in the sand ranging from 1-12 inches deep. During high tide, they live near soil surface (1 inch deep) in order to filter their food such as plankton and detritus suspending in water.

Solen regularis could be found at high density when the percentage of sand was more than 80 % in the soil composition and lower density could be found in muddy soil. Average growth rate of *Solen regularis* was found about 1.2 mm./month. The optimum condition for *Solen regularis* depends on many factors, such as the amount of dissolved oxygen, nitrite, ammonia, water temperature and pH. If pH reached 9.1, *Solen regularis* would died (Veeravaitaya, 2007). Its classification as follows (Zipcodezoo, 2008d):

Domain: Eukaryota (Whittaker and Margulis, 1978)

Kingdom: Animalia (Linnaeus, 1758) - animals

Subkingdom: Bilateria (Hatschek, 1888), Cavalier (Smith, 1983)

Branch: Protostomia (Grobbs, 1908)

Infrakingdom: Lophotrochozoa

Superphylum: Eutrochozoa

Phylum: Mollusca (Linnaeus, 1758), Molluscs (Cuvier, 1795)

Class: Bivalvia (Linnaeus, 1758) - Bivalves

Subclass: Metabranhia

Superorder: Eulamellibranchia

Order: Veneroida (H. and A. Adams, 1856)

Superfamily: Solenoidea

Family: Solenidae (Lamarck, 1809)

Genus: *Solen* (Linnaeus, 1758)

Specific name: *regularis* - Dunker

Scientific name: - *Solen regularis* Dunker



Figure 2.8 Razor clam (*Solen regularis*)
(Nairoburoo, 2007)

2.2.2 *Paphia undulata*

Paphia undulata (Figure 2.9) was found in the Gulf of Thailand and Andaman Sea. Also known as sand clams: moke-kaung (Burma); him (China); kere (Indonesia); tepeh (Malaysia); bigatan (Philippines); soup clams: dendang (Indonesia); remis (Malaysia); hoy lai (Thailand) (Passmore, 1991). In general, *Paphia undulata* grounds consist of consolidated mud substrates within 3 to 7 km. distance from the shoreline. It is found in deeper water, between 1-8 m. depth and dig in the sand about 20 cm. *Paphia undulata* has triangular-shape with an intricate pattern of dark brown, zigzag markings over the yellowish shell. Its classification as follows (Zipcodezoo, 2008b):

Domain: Eukaryota (Whittaker and Margulis, 1978)
 Kingdom: Animalia (Linnaeus, 1758) - animals
 Subkingdom: Bilateria (Hatschek, 1888), Cavalier (Smith, 1983)
 Branch: Protostomia (Grobber, 1908)
 Infrakingdom: Lophotrochozoa
 Superphylum: Eutrochozoa
 Phylum: Mollusca (Linnaeus, 1758), Molluscs (Cuvier, 1795)
 Class: Bivalvia (Linnaeus, 1758) - Bivalves
 Subclass: Metabranhia
 Superorder: Eulamellibranchia
 Order: Veneroida (H. and A. Adams, 1856)
 Superfamily: Veneroidea
 Family: Veneridae (Rafinesque, 1815)
 Subfamily: Tapetinae
 Genus: *Paphia* (Röding, 1798)
 Specific name: *undulata*
 Scientific name: - *Paphia undulata*



Figure 2.9 Undulated surf clam (*Paphia undulata*)
(Eurobrokers and Co. srl., 2007)

2.2.3 *Anadara granosa*

Anadara granosa (Figure 2.10) is a shellfish that provides a good protein resource. It is widely available and commonly eaten in the Southeast Asia countries. *Anadara granosa* farming is extensively distributed in the eastern and southern coast of Thailand. It is typically found in the tidal zone at the water's edge at a mean distance from the shore about 1-2 km. and live under the muddy soil about 1-12 inches. Its classification as follows (Zipcodezoo, 2008a):

Domain: Eukaryota (Whittaker and Margulis, 1978)
Kingdom: Animalia (Linnaeus, 1758) - animals
Subkingdom: Bilateria (Hatschek, 1888), Cavalier (Smith, 1983)
Branch: Protostomia (Grobden, 1908)
Infrakingdom: Lophotrochozoa
Superphylum: Eutrochozoa
Phylum: Mollusca (Linnaeus, 1758), Molluscs (Cuvier, 1795)
Class: Bivalvia (Linnaeus, 1758) - Bivalves
Subclass: Metabranhia
Superorder: Filibranchia
Order: Pteriomorpha
Superfamily: Arcoidea
Family: Arcidae (Lamarck, 1809)
Subfamily: Anadarinae
Genus: *Anadara* (Gray, 1847)
Specific name: *granosa* - (L., 1758)
Scientific name: - *Anadara granosa* (L., 1758)



Figure 2.10 Blood cockle (*Anadara granosa*)
(Bishogai, 1997)

2.2.4 *Perna viridis*

Perna viridis (Figure 2.11) is widely distributed in the Indo-Pacific from the Persian Gulf to the Philippines and Hong Kong. Introduced to Japan, Fiji, Tahiti, Queensland (Australia), Trinidad, Venezuela, Jamaica, and Florida. The *Perna viridis*'s sexual development was shown to be affected by temperature, it will be killed by the cold temperature. *Perna viridis* ranges from 8 to 10 cm. in length.

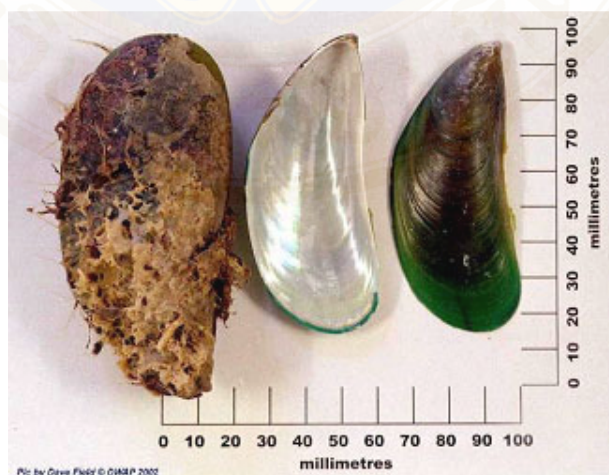


Figure 2.11 Green mussel (*Perna viridis*)
(Bloggang, 2005)

Mussel is a filter feeder that feeds on phytoplankton, zooplankton and suspended organic materials. The growth rate is influenced by the availability of food, temperature, water currency, mussel's age, and caging. The artificial cage culturing can prevent entry of predators and barnacles but slows down the mussel's growth rate. Its classification as follows (Zipcodezoo, 2008c):

Domain: Eukaryota (Whittaker and Margulis, 1978)
Kingdom: Animalia (Linnaeus, 1758) - animals
Subkingdom: Bilateria (Hatschek, 1888), Cavalier (Smith, 1983)
Branch: Protostomia (Grobbs, 1908)
Infrakingdom: Lophotrochozoa
Superphylum: Eutrochozoa
Phylum: Mollusca (Linnaeus, 1758), Molluscs (Cuvier, 1795)
Class: Bivalvia (Linnaeus, 1758) - Bivalves
Subclass: Metabranhia
Superorder: Filibranchia
Order: Pteriomorpha
Superfamily: Mytiloidea
Family: Mytilidae (Rafinesque, 1815) - Marine Mussels
Subfamily: Mytilinae
Genus: *Perna* (Philipsson, 1788)
Specific name: *viridis* - (Linnaeus, 1758)
Scientific name: - *Perna viridis* (Linnaeus, 1758)

2.3 Analytical Methods for TBT

The materials of marine origin can be divided roughly into three categories: water, sediment and biological materials. Generally there are four successive stages to analysis as follows:

2.3.1 Extraction

Extraction is necessary for sediments and aquatic biota tissues and can also be applied in the analysis of water samples. In the case of solid samples, there are many extraction techniques such as Soxhlet, ultrasound, microwave, supercritical fluid and pressurized solvent systems. Ultrasonication method is the most commonly used, whereas, microwave energy and pressurized solvent extraction have begun to be widely applied as well. In the last years, accelerated solvent extraction (ASE) is popularly used for organotin speciation. The main advantages of ASE are the high sample throughput, significantly lower solvent consumption, lower cost, and the high potential of being used routinely (Brunori *et al.*, 2006).

In the case of water samples, the applied extractants can be solvents or solid phases. The main advantages of liquid-liquid extraction (LLE) are the high efficiency, reproducibility in recovery and high enrichment factors. However, it may be replaced by adsorption onto silica gel bonded with C₁₈ aliphatic chains known as solid phase extraction (SPE) (Matthias *et al.*, 1986a, b ; Humphrey and Hope, 1987). Brunori, *et al.* (2006) reported that the advantages of SPE over LLE are as it is easy to handle, adaptable to field analysis, it is of lower cost, allows high routine, and presents a very low potential health hazard.

2.3.2 Derivatization

According to WHO (1990), mono-, di-, and tri-butyltins are not sufficiently volatile to perform their separation on gas chromatography (GC), hence, it is necessary to produce more volatile organotin compounds to allow better separation. Derivatization methods are based on the alkylation or hydridization reactions. Alkyl derivatives formation by using of Grignard's reagent (RMgBr) is simpler and preferred for sediments and biota samples.

Hydride generation with sodium borohydride (NaBH₄) is a procedure widely used for organotin determination. Brunori, *et al.* (2006) reported that hydridization is more suitable for dilute aqueous samples such as water than the alkylation method because it shows high yields of derivatization. On the other hand, hydridization is strongly influenced by the high metal concentration of the matrices which affect the efficiency and reproducibility of the derivatization.

2.3.3 Separation (Clean-up)

High performance liquid chromatography and gas chromatography are more frequently used for separation than thin-layer chromatography because of its low sensitivity (WHO, 1990).

Silica gel is the adsorbent most popular used in order to purify the organotin before it was directly injected in the chromatographic system, other adsorbent are: Alumina, Alumina-silica, amino, C₁₈ and Floril. Consequently the elution step using hexane or hexane-diethyl ether mixtures was performed.

2.3.4 Analysis

There are several methods available for measuring TBT in environmental samples to detection limits of 0.2 to 5 ng/L in water and 5 to 30 µg/kg in sediment and biota samples (WHO, 1990). The most commonly applied techniques have been based on gas and liquid chromatographic separation followed by different types of detectors as below:

2.3.4.1 Flame photometric detection (FPD), detection is based on tin molecular emission in a hydrogen-air flame. The most commonly selected radiance is approximately 610 nm. The main advantages are the increased sensitivity and lower matrix dependency.

2.3.4.2 Flame atomic absorption (AA) spectrometer or flameless atomic absorption (FAA) spectrometer using a graphite furnace. In the AAS systems tin is quantified at 286.3 or 224.4 nm.

2.3.4.3 Flame ionization detector (FID) is the most sensitive gas chromatographic detector for hydrocarbons such as butane or hexane. With the detection limitation in the low picogram or femtogram range, the FID is the most widely and successfully used gas chromatographic detector for volatile hydrocarbons and many carbon containing compounds.

2.3.4.4 Electron capture detector (ECD) is one of the most sensitive gas chromatography detectors available. The ECD uses a radioactive Beta emitter (electrons) to ionize some of the carrier gas and produce a current between a biased pair of electrodes. The ECD is as sensitive as the FID but has a limited dynamic range and finds its greatest application in analysis of halogenated compounds.

2.3.4.5 Mass spectrometry (MS) is useful for precise identification of the substance but has limited sensitivity. MS uses the difference in mass-to-charge ratio of ionized atoms or molecules to separate them from non-target chemicals. MS has three essential modules as shown in Figure 2.12 (Wikipedia, 2008). The process starts with an ion source, which transforms the molecules in a sample into ionized fragments. Follow by a mass analyzer, which sorts the ions by their masses by applying electric and magnetic fields. End up with a detector, which measures the value of some indicator quantity and thus provides data for calculating the abundances of each ion fragment present.

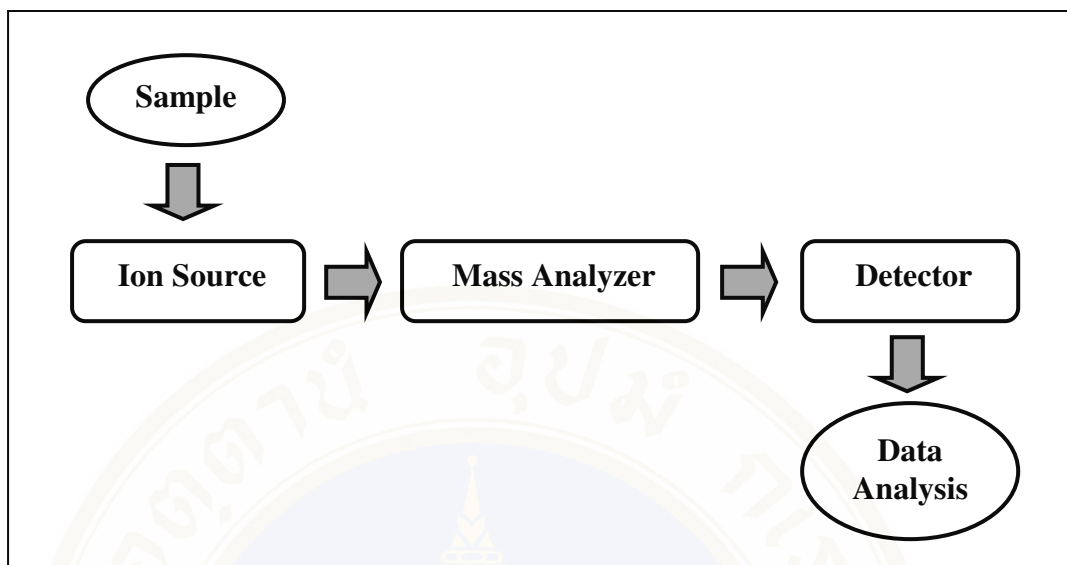


Figure 2.12 MS schematic

Brunori, *et al.* (2006) summarized the most applied extractants, derivatizing reagents, and instrumental techniques for the determination of organotin species in marine biota, marine water, and marine sediment in Table 2.7, 2.8, and 2.9, respectively.

Table 2.7 Organotins determination techniques for marine biota (Brunori *et al.*, 2006)

Extraction	Derivatization	Analysis
• Acid & polar solvent - HCl, acetic acid/ MEOH • Acid & varying polarity solvent - HCl/toluene, diethyl ether, ethyl acetate • ASE - Sodium acetate & acetic acid/MeOH	• Grignard; - pentylation	• GC-MS • GC-FPD • HPLC

Table 2.8 Organotins determination techniques for marine water (Brunori *et al.*, 2006)

Extraction	Derivatization	Analysis
• LLE; - DCM - Hexane - Toluene - Diethylether - Acetone - Acetonitrile - Mixtures • SPE	• Hydride generation • Grignard; - pentylation	• GC-MS • GC-FPD

Table 2.9 Organotins determination techniques for marine sediment (Brunori *et al.*, 2006)

Extraction	Derivatization	Analysis
• ASE - Acetic acid/MeOH & tropolone • SPE	• Hydride generation • Grignard; - pentylation	• HPLC • GC-AAS • GC-MS • GC-FPD • GC-FID

2.4 Previous Studies

In order to run the vessel ship, the speed per energy consumption unit is the important. It is considered that aquatic organisms, such as barnacles, seaweeds, or tubeworms on a boat's bottom produce roughness that increase turbulent flow and can increase fuel consumption of about 0.3-1.0%. The primary marine antifouling paint was based on cuprous (copper) oxide which is the effective biocide. In the early 1970s, this compound is no longer used in antifoulant paints because of its toxicity to the environment. Then, a widespread use of TBT-based antifouling paints was highly demand. TBT-based paint on hull surfaces was designed to get a constant TBT leach rate of $1.6 \mu\text{g}(\text{Sn})/\text{cm}^2$ per day. The leach rate of freshly painted surfaces was about $6 \text{ mg}(\text{Sn})/\text{cm}^2$ per day. The monitoring was done in a harbor for 3 days, a commercial ship could release more than 200 g of TBT into water and could be high up to 600 g in the freshly painted ship (Champ and Seligman, 1996).

Harino, *et al.* (1997) studied the degradation of the tributyltin compounds by the microorganisms in water and sediment collected from the harbor area of Osaka City, Japan. The ranges of TBT contamination in surface water were from 0.002 to 0.033 mg/L, while TBT in sediment samples were measured in the range of 0.002 to 0.966 mg/kg (dry weight). The highest TBT concentration in surface water and sediment were found in a marina and the zone of poor flushing. The degradation of TBT to DBT and MBT by microorganisms from water and sediment was compared. The microorganisms in water degraded TBT faster than that from sediment because the stability of TBT in sediment is higher than in water.

Kan-atireklap, *et al.* (1997b) investigated the contamination of butyltin compounds in sediment from the coastal areas in Thailand. The ranges of MBT, DBT, and TBT in surface sediments ranging from 7 to 410, 2 to 1900, and 4 to 4500 ng/g (dry weight basis), respectively. The high TBT concentrations in sediments were found in far seas vessels or commercial shipping harbors, such as Chao Phraya River, Samut Prakan, Paknam, Chumporn and Ao Makham, Phuket. However, the low TBT concentrations in sediments were found in small fishing boat piers and coastal mariculture areas.

Arnold, *et al.* (1998) conducted experiments on determination of organotin compounds in water, sediments, and sewage sludge using Perdeuterated internal standards, Accelerated solvent extraction (ASE), and Large-Volume-Injection GC-MS. Two new methods were presented for simultaneous identification and quantification of organotin compounds. For aqueous samples aqueous ethylation followed by liquid-liquid extraction (LLE) or solid phase extraction and large volume injection GC-MS. The solid sediment samples were freeze-dried and kept at -25°C until analysis (within 2 months). Sodium acetate/acetic acid is used as solvent for the sample with NaBEt₄ used to derivatize and extract the organotins. This is similarly applied on the aqueous sample. It is later analysed by large-volume injection GC-MS.

Tanabe, *et al.* (2000) investigated the contamination of butyltin and organochlorine compounds in green mussels along the coastal areas of Thailand, Philippines, and India. High concentrations of butyltins in green mussel were found at locations with intensive boating activities and coastal aquaculture facilities, implying the usage of TBT as a biocide in antifouling paints used on boat hulls and in marine

aquaculture. The results showed that the concentration of butyltins in green mussels from Thailand were found to be higher in mussels from aquaculture areas than those from high fishing boat activity areas. It is considered that butyltins contamination levels in Asian developing countries were lower than those in developed nations. However, considering the fact that the unregulated usage of organotins in developing countries in Asia and the increasing demand for antifouling paints in the region, butyltins contamination in the aquatic environment may increase in the future.

A study of the optimization and validation by SPE-CGC-MSD of the analysis of tributyltin in environmental samples was conducted by Nogueira, *et al.* (2001). The SPE-CGC-MSD methodology of TBT analysis has the advantage on well performance, easy to operate, and fast. The results showed a value of 50 ± 18 ng/g for TBT in the sediment matrices assays which, according to European Co-operation for Accreditation of Laboratories guidance for interlaboratory comparisons, led to a deviation normalized lower than 0.2. Moreover, recoveries for the in-house standard aqueous matrices assays are higher than 85%, showing that this methodology is validated, due to the high accuracy reached.

It is known that the accumulation of TBT can cause imposex in gastropods. Bech (2002) reported that imposex in *Thais distinguenda* and *Thais bitubercularis* collected from the west coast of Thailand have been increased significantly from 1996 to 2000. The less sensitive species, such as *Morula musiva*, *Morula granulate*, *Morula margariticola*, and *Thais rufotincta* only developed imposex at the three main areas of high shipping activities. The use of *Thais distinguenda* and *Thais bitubercularis* are recommended as indicators of TBT in Southeast Asia because of their sensitivity and wide distribution in the region.

Nemanič, *et al.* (2002) investigated organotin compounds in the marine environment of the Bay of Piran, Northern Adriatic Sea. Field sampling of water samples was taken place in summer and autumn at 8 locations. Mussels (*Mytilus galloprovincialis*) were collected in summer at 3 locations. Gas chromatograph (GC) with mass selective detector (MSD) was introduced and validated applying pentylation with a Grignard reagent. The highest concentrations of TBT in water samples ranged from 500 to 630 ng/L (as Sn) in summer and from 180 to 230 ng/L (as Sn) in autumn.

The highest TBT accumulation in mussels ranged from 500 to 3500 ng/g (as Sn) (dry weight).

Bortoli, *et al.* (2003) determined the contamination of butyltins and phenyltins in biota and sediments from the Lagoon of Venice, Italy. Sediments and aquatic organisms were collected at 12 locations in the lagoon for the analysis. The results showed that organotin contamination in sediments is at lower levels, ranging from 2.5 ± 0.1 to 84 ± 1 ng/g (dry weight) for butyltin compounds and from 0.8 ± 0.2 to 7 ± 1 ng/g (dry weight), for phenyltin compounds, than in organisms, where the highest concentrations in filter feeders like mussels were found from 60 ± 3 to $7,632 \pm 148$ ng/g (dry weight) for butyltin compounds and from 0.80 ± 0.01 to 4005 ± 121 ng/g (dry weight) for phenyltin compounds. The contamination of TBT in biota samples are below the Tolerable Average Residue Level (TARL), except in mussels and clams at some sampling stations.

Murai, *et al.* (2005) studied the status of butyltin contamination along the coasts of western Japan in 2001, 11 years after the 1990 regulations on the use of TBT in aquaculture and on boats. Seawater and *Caprella* spp. samples were collected from the western part of Seto Inland Sea, Uwa Sea and Uranouchi Bay in western Japan during March to September, 2001. The average concentration of butyltins, ranging from 8.2 ± 9.2 (SD) ng MBT/L, 3.3 ± 3.0 ng DBT/L, and 9.0 ± 7.0 ng TBT/L. This indicates that the observed levels of TBT in Seto Inland Sea and adjacent waters have contributed to the reduction in the survival rate of *Caprella* spp. Although, the partial ban on TBT usage was implemented, the concentration of TBT in seawater still increase and tend to reduce the production of tropic fishes.

Murata, *et al.* (2008) determined the contamination of organotin compounds in the liver of adult sea otters (*Ennhydra lutris*) found dead along the coasts of California, Washington, and Alaska in the USA and Kamchatka (Russia). Total concentrations of organotin compounds in sea otters from California were found in the ranges of 34 to 4100 ng/g (wet weight). Although organotin compound concentrations in sea otters were decreasing since the 1990s, continuing high adult mortality with infectious disease is still concerned.

CHAPTER III

MATERIALS AND METHODS

3.1 Research Procedures

The overall procedures involved, preparation of equipment, method validation, collection of samples, preparation of samples, monitoring of TBT concentration in samples, and data analysis. After the analytical procedures were designed, the methods were validated in order to obtain the optimum condition and the accuracy of the processes. Subsequently, the analysis of TBT in shellfish, marine water, and sediment was performed. In addition, TBT contaminations in shellfish were used to evaluate the hazard to human health (Figure 3.1).

3.2 Experimental Location

Experimental site of this research was located at the laboratory of Civil Engineering Department, Faculty of Engineering, Mahidol University, Salaya Campus. The operation of GC-MS instrument was done at The Center for Environmental Health, Toxicology, and Management of Chemicals under Higher Education Development Project of the Commission on Higher Education, Ministry of Education, Faculty of Science, Mahidol University, Phayathai Campus.

3.3 Materials

3.3.1 Materials used in method validation would include the following:

3.3.1.1 Glass filter paper

3.3.1.2 Deionized water

3.3.2 Materials used in sample analysis would include the following:

3.3.2.1 Four shellfish species: razor clam, undulated surf clam, blood cockle, and green mussel

3.3.2.2 Marine water

3.3.2.3 Sediment

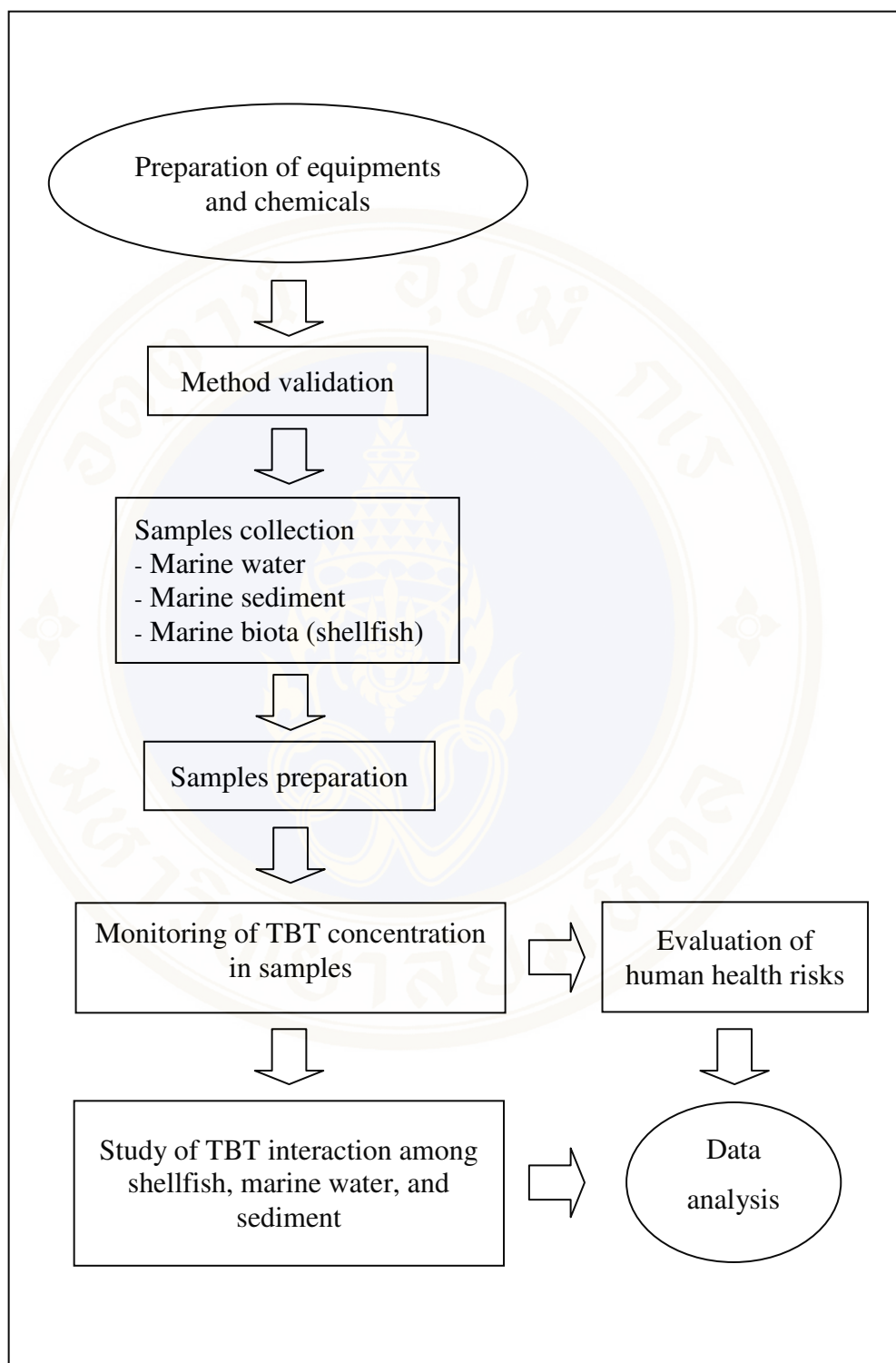


Figure 3.1 Schematic diagram of research methodology

3.4 Instruments

The details of various equipments used in this study are given below:

3.4.1 Accelerated Solvent Extractor (ASE)

Accelerated solvent extractor (Dionex, ASE200) (Figure 3.2) is an extraction technique combining high temperatures and pressures with liquid solvents. ASE uses conventional liquid solvents at elevated temperatures and pressures to increase the efficiency of the extraction process. The advantages of ASE are the high sample throughput, low solvent consumption, and high potential of being used routinely. Following diagram (Figure 3.3) shows the extraction steps employed by ASE200.



Figure 3.2 Accelerated solvent extractor (ASE200)

3.4.2 Gas Chromatograph-Mass Spectrometer (GC-MS)

Gas chromatograph-mass spectrometer (Figure 3.4) is actually two techniques that are combined to form a single method of analyzing mixtures of chemicals. The GC instrument (Agilent Technologies, 6890 N) is effective in separating compounds into their various components, whereas, the MS instrument (Agilent Technologies, 5975 Inert XL) provides specific results of target chemicals. GC-MS is widely used for confirmation testing of substances, for instance, drug testing, manufacturing quality control, and environmental testing to evaluate samples containing the micropollutants.

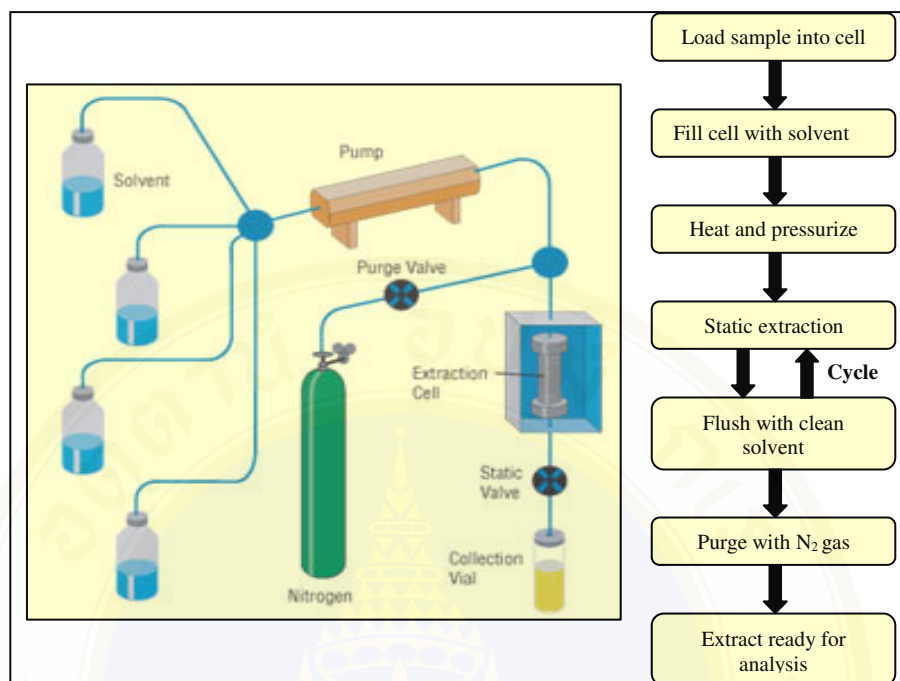


Figure 3.3 ASE schematic



Figure 3.4 Gas Chromatograph-mass spectrometer

(Shimadzu, 2007)

3.5 Study Design

Part I: Validation of analytical methods. Method validation had been done for 3 times ($n=3$) in order to confirm the analytical procedures employed for trial test are suitable for the real samples. The procedures are described as follows:

3.5.1 1.2 μm non-TBT glass fiber filter papers and deionized water were spiked with TBT standard solution and used as samples.

3.5.2 Extraction conditions, clean up processes, and GC-MS conditions for TBT analysis in shellfish, marine water, and sediment samples were optimized.

3.5.3 TBT analytical methods were verified for 3 times before the real samples performance.

Part II: Monitoring of TBT concentration in shellfish samples. This part was carried out in order to determine the concentration of TBT in shellfish samples available in two major seafood markets. Maha Chai Market, Samut Sakron Province, one of Thailand's largest traditional fresh seafood markets and Don Hoi Lot Market, a sandbar off the coast of Samut Songkhram Province. All samples were collected in winter season (November, 2008) in Thailand. The procedures are described as follows:

3.5.4 Undulated surf clam, blood cockle, and green mussel were collected from Maha Chai Market.

3.5.5 Razor clam, blood cockle, and green mussel were collected from Don Hoi Lot Market.

3.5.6 Samples were prepared and analyzed by using GC-MS.

Part III: The determination of TBT concentration in shellfish, marine water, and sediment samples taken from the place of origin. All samples were collected in rainy season (May-June, 2010) along coastal areas of Thailand. Blood cockle from Bang Khun Sai Subdistrict, Ban Laem District, Phetchaburi Province. Razor clam from Bang Ja Krong Subdistrict, Mueang District, Samut Songkhram Province. Undulated surf clam from Mae Klong River, Samut Songkhram Province. Green mussel from Phanthai Norasing Subdistrict, Mueang District, Samut Sakron Province (Figure 3.5). The procedures are described as follows:

3.5.7 Shellfish, marine water, and sediment samples were collected from the cultural places.

3.5.8 Samples were prepared and analyzed by using GC-MS.

Part IV: Data analysis by using primary and secondary data. The highest TBT concentration of each shellfish species was used in the evaluation of human health risks.

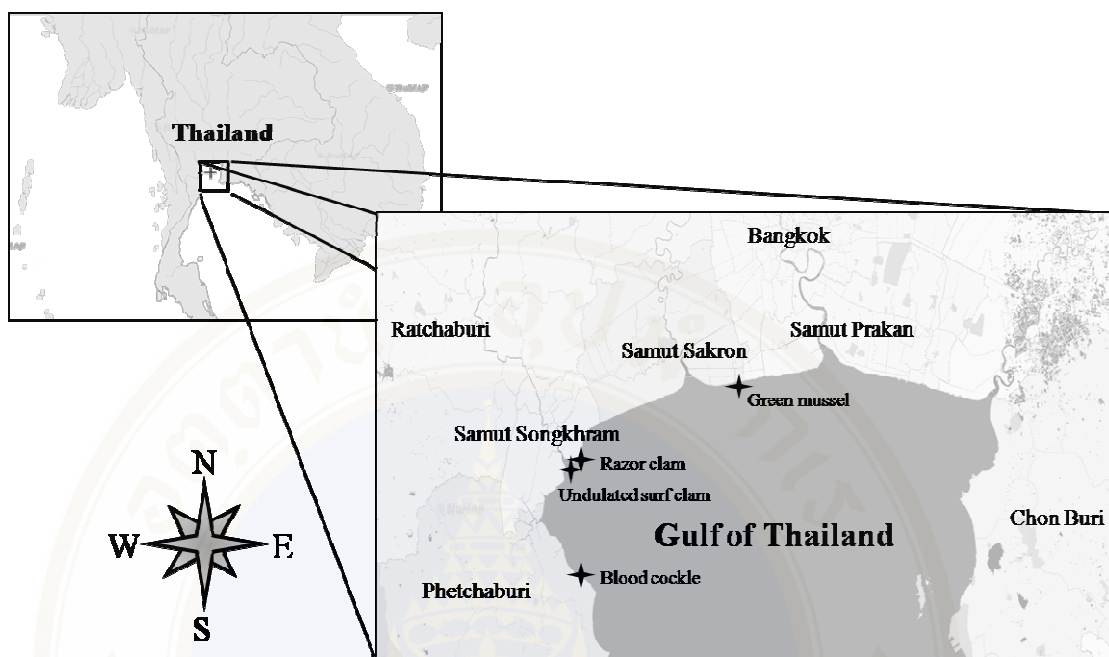


Figure 3.5 Aquaculture areas of 4 shellfish species; undulated surf clam, blood cockle, green mussel, and razor clam

3.6 Analytical Methods

Analytical methods were optimized and validated before the analysis of real samples; shellfish, water, and sediment. The analytical processes are described as follows: firstly, the spiked glass filter papers were extracted by ASE to obtain the organotin compounds in liquid phase and followed by liquid-liquid extraction (LLE) technique, whereas, marine water samples were directly extracted by LLE technique. Secondly, the samples were derivatized and cleaned up to prepare the volatile organotins for GC analysis. Lastly, organotin compounds were determined by GC-MS. In addition shellfish, marine water, and sediment samples were collected from the interesting aquaculture areas and kept in the appropriate containers until the analysis. The methods for marine samples were obtained from the validation. Glassware used in this research, were washed with detergent, soaked in 10 % nitric acid (65 % HNO_3 , purchased from Merck) for a few hours, rinsed with distilled water, followed by methanol, and deionized water in order to refrain any TBT contamination.

3.6.1 Optimization and Validation of Experimental Procedures

3.6.1.1 Elution

10 ml of 250 ng ml⁻¹ TBT standard solution were prepared from tributyltin (97 % TBTCI, purchased from Wako) standard stock solution (1 mg ml⁻¹ as Sn). The stock solution was prepared in hexane (purchased from Fisher Scientific) and stored in dark bottle at 4 °C. TBT standard solution in a 50 ml conical flask with rubber septum was pentylation with Grignard reagent in order to enhance more volatile compounds. Derivatization was carried out with the addition of 1.5 ml of pentyl-magnesium bromide 2 mol l⁻¹ in diethyl ether (PeMgBr, purchased from Sigma-Aldrich) under nitrogen condition, the organic phase was stirred in 40 °C water bath by magnetic stirrer for 45 min.

The excess Grignard reactant was destructed with 7 ml of 2 mol l⁻¹ sulfuric acid (98 % H₂SO₄, purchased from Ajax Finechem), stirred for 1 min and transferred to 100 ml separatory funnel. The derivatized organotin was subsequently washed with 20 ml of deionized water (18.2 MΩ, Millipore system) in order to dilute any acidic residues. The solution was left for phase separation after that the water layer was discarded. Then the sample was reduced to 5 ml under nitrogen gas and loaded into the silica gel column (1 cm id x 30 cm). As a clean up step, a glass column was wet-packed in hexane with 6 g of activated silica gel (obtained from Merck) and 1 g of activated sodium sulfate anhydrous (Na₂SO₄, purchased from Fluka) on the top (incubated at 180 °C for 12 hr). 5 ml of the eluate were placed into 25 ml beaker. Subsequently sample was eluted with 10 ml of (9:1) hexane:diethyl ether (purchased from Lab-Scan) mixture and placed into a different 25 ml beaker. Elution was done for 3 times. After that each of the solutions was reduced the volume to 1 ml and transferred to a GC vial for analysis by GC-MS.

An Agilent 6890 N Network gas chromatograph coupled to an Agilent 5975 Inert XL MSD quadrupole mass spectrometer (Agilent Technologies). The GC system was equipped with a splitless capillary injector, and a HP-5 capillary column (30 m x 0.25 mm I.D. x 0.25 μm film thickness). Helium was employed as carrier gas with a constant flow of 0.8 ml min⁻¹, average velocity 33 cm sec⁻¹, pressure 6.8 psi, and the injection volume is 1 μl. The optimum GC-MS conditions (Table 3.1) are based on the procedure reported by Kunprasert (2003).

The oven temperature was programmed as follows: 80 °C held for 1 min with a heating rate of 20 °C min⁻¹ to 200 °C, from 200 to 221 °C at 3 °C min⁻¹ and then held at 300 °C for 6 min to clean out the column. The injector temperature was held at 280 °C, and heater set point at 280 °C. The MSD operated with the electron ionization (EI) ion source conditions were: electron energy 70 eV and temperature 230 °C. The quadrupole temperature was set at 150 °C. In multiple selected ion monitoring (MS-SIM) the most abundant tin isotopic peaks (m/z: 179 and 305) of the organotin species were applied in each retention time windows, with a dwell time of 100 sec. A schematic diagram of elution validation of TBT analytical procedures was shown in Figure 3.6.

Table 3.1 Operating conditions for GC-MS

Instrument:	GC-MS
Column:	HP-5 (30 m x 0.25 mm I.D. x 0.25 µm)
Carrier gas:	He
Inlet:	Splitless
Temperature program:	80 °C, hold for 1 min to 200 °C Rate : 20 °C min ⁻¹ 200 °C to 221 °C Rate : 3 °C min ⁻¹ 221 °C to 300 °C, hold for 6 min
Injection volume:	1 µl
Injector temperature:	280 °C
Interface temperature:	280 °C
Gas flow:	1 ml min ⁻¹
Detector:	Mass spectrometer
Mass range:	50-450
Scan:	Solvent delay : 5 min
Sim:	Ion : 179, 305 Dwell time : 100 sec

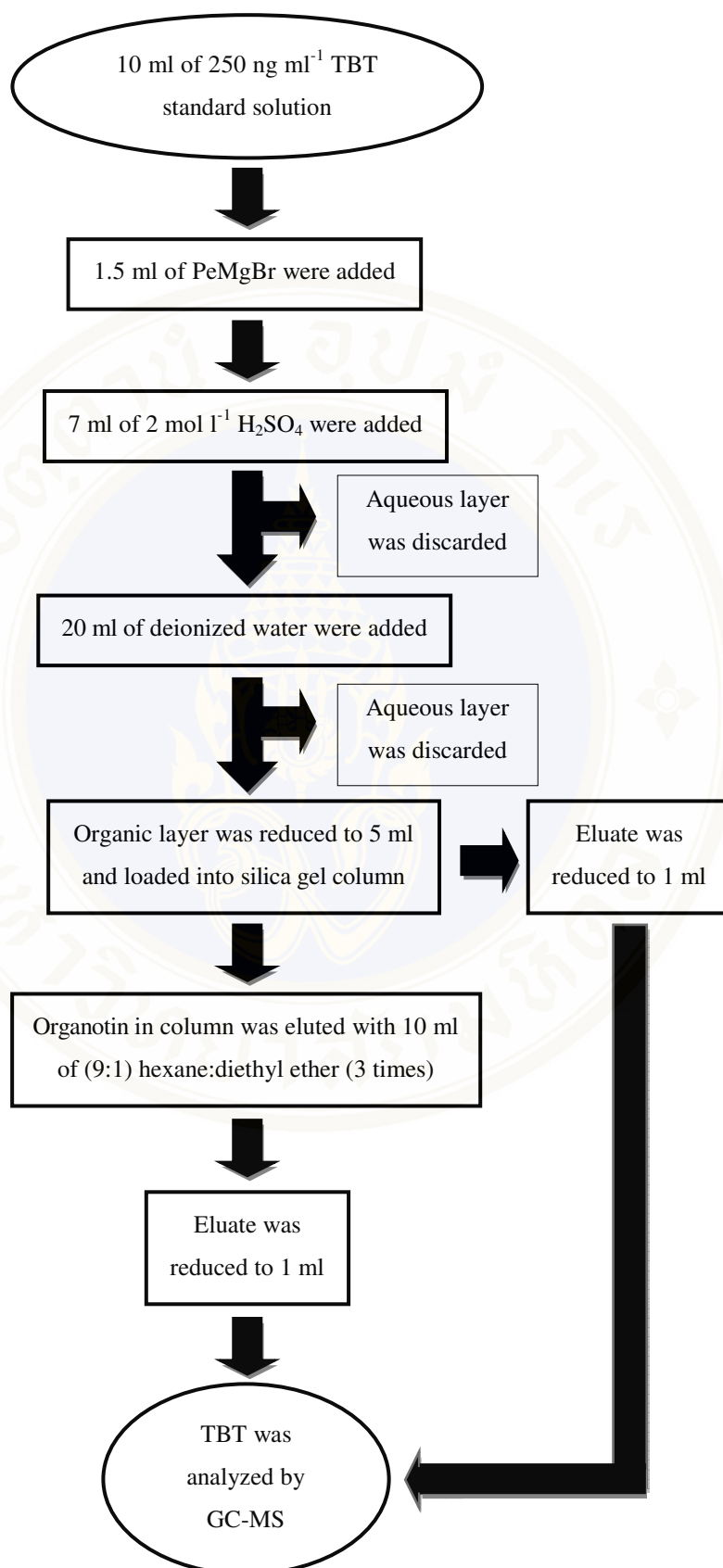


Figure 3.6 A schematic diagram of elution validation of TBT analytical procedures

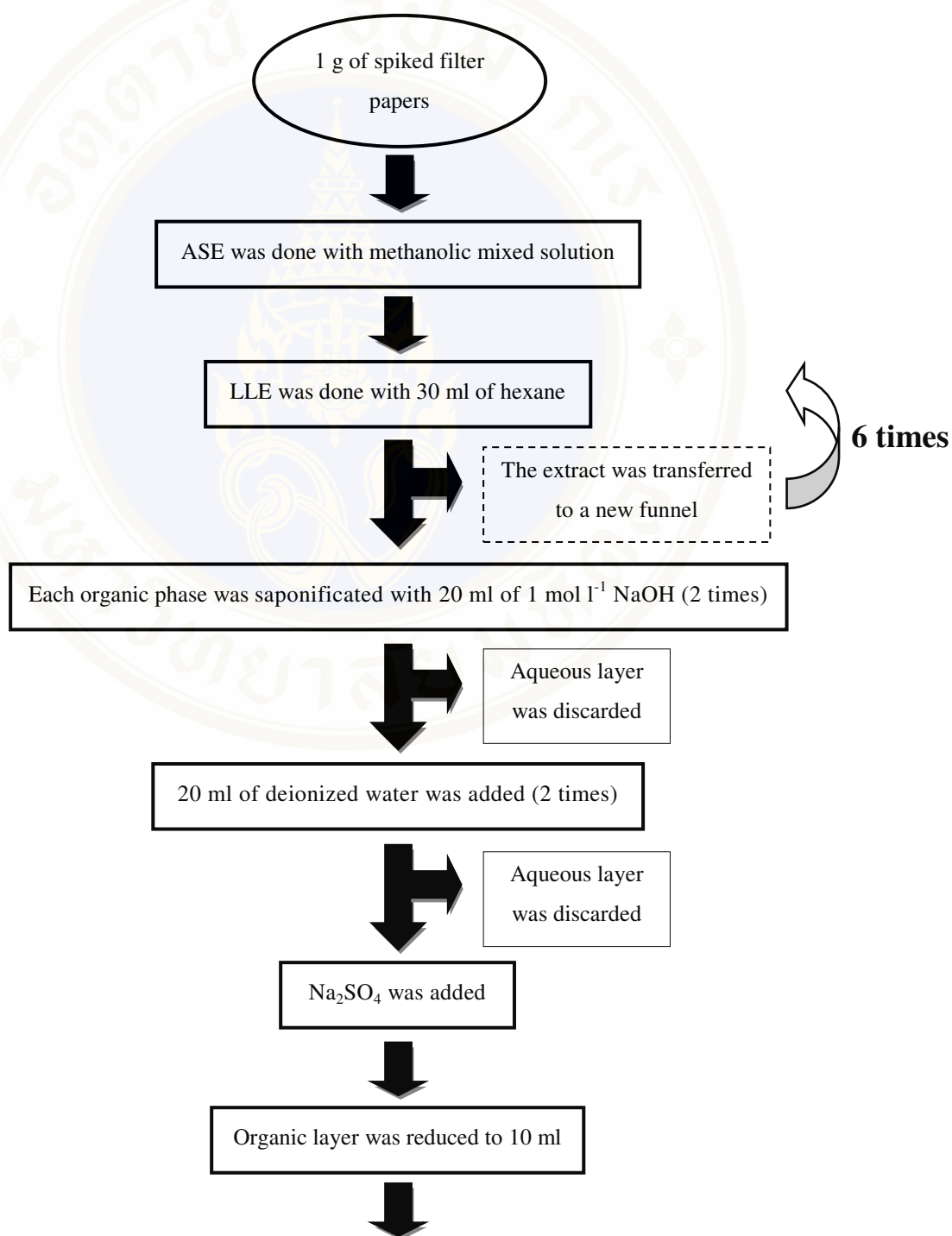
3.6.1.2 Liquid-liquid extraction

1.2 μm non-TBT glass fiber filter papers (GF/C, purchased from Whatman) were weighed approximately 1 g and spiked with a 500 ng ml^{-1} concentration of TBT standard solution. The spiked filter papers were cut into small pieces and placed in an 33 ml ASE extraction cell. The ASE200 accelerated solvent extractor (Dionex) was used for sample extraction. The optimum ASE conditions are based on the method reported by Wasik and Ciesielski (2004). The following extraction parameters were used: temperature 125°C , pressure 800 psi, pre-heat time 6 min, heat time 6 min, static extraction time 3 min, flush volume 20 %, purge time 100 s, and 4 static extraction cycles. Solvent composition: 900 ml of methanol (MeOH, purchased from Merck), 100 ml of deionized water, 1 mol of acetic acid (CH_3COOH , obtained from Merck), 1 mol of sodium acetate ($\text{C}_2\text{H}_3\text{NaO}_2$, purchased from Carlo Erba), and 0.3 g of tropolone (98 % Tropolone, obtained from Acros Organics).

35 ml of extract was transferred to 100 ml separatory funnel and extracted about 10 min with 30 ml of hexane for 6 times. Each organic layer in separatory funnel was saponificated about 1 min with 20 ml of 1 mol l^{-1} sodium hydroxide (NaOH, purchased from Ajax Finechem) for 2 times in order to eliminate lipid. After the solution was left in a room temperature for 10 min to allow phase separation, the aqueous phase was discarded. Subsequently the organic layer was washed for 2 times with 20 ml of deionized water to dilute any basic residues from saponification step. After the aqueous layer was released, the moisture in organic phase was removed by an additional of sodium sulfate anhydrous. The extract was concentrated by heating with hot plate until the volume is about 10 ml and transferred to a 50 ml conical flask with rubber septum.

The reduced organic phase was allowed to react with 1.5 ml of pentyl-magnesium bromide 2 mol l^{-1} in diethyl ether under nitrogen condition, the organic phase was stirred in 40°C water bath by magnetic stirrer for 45 min. The excess pentyl-magnesium bromide was destructed with 7 ml of 2 mol l^{-1} sulfuric acid, stirred for 1 min and transferred to 100 ml separatory funnel. After that pentylated organotin was washed with 20 ml of deionized water. Then the organic layer was evaporated with nitrogen to exactly 5 ml and loaded into the silica gel

column (6 g of activated silica gel and 1 g of activated sodium sulfate anhydrous). Subsequently sample was eluted with a 20 ml of (9:1) hexane:diethyl ether mixture. After that the solution was reduced the volume to 1 ml and transferred to a GC vial for analysis. 1 μl was injected into GC-MS, the optimum conditions were obtained from Kunprasert (2003). Schematic presentation of liquid-liquid extraction validation of TBT analytical procedures was shown in Figure 3.7.



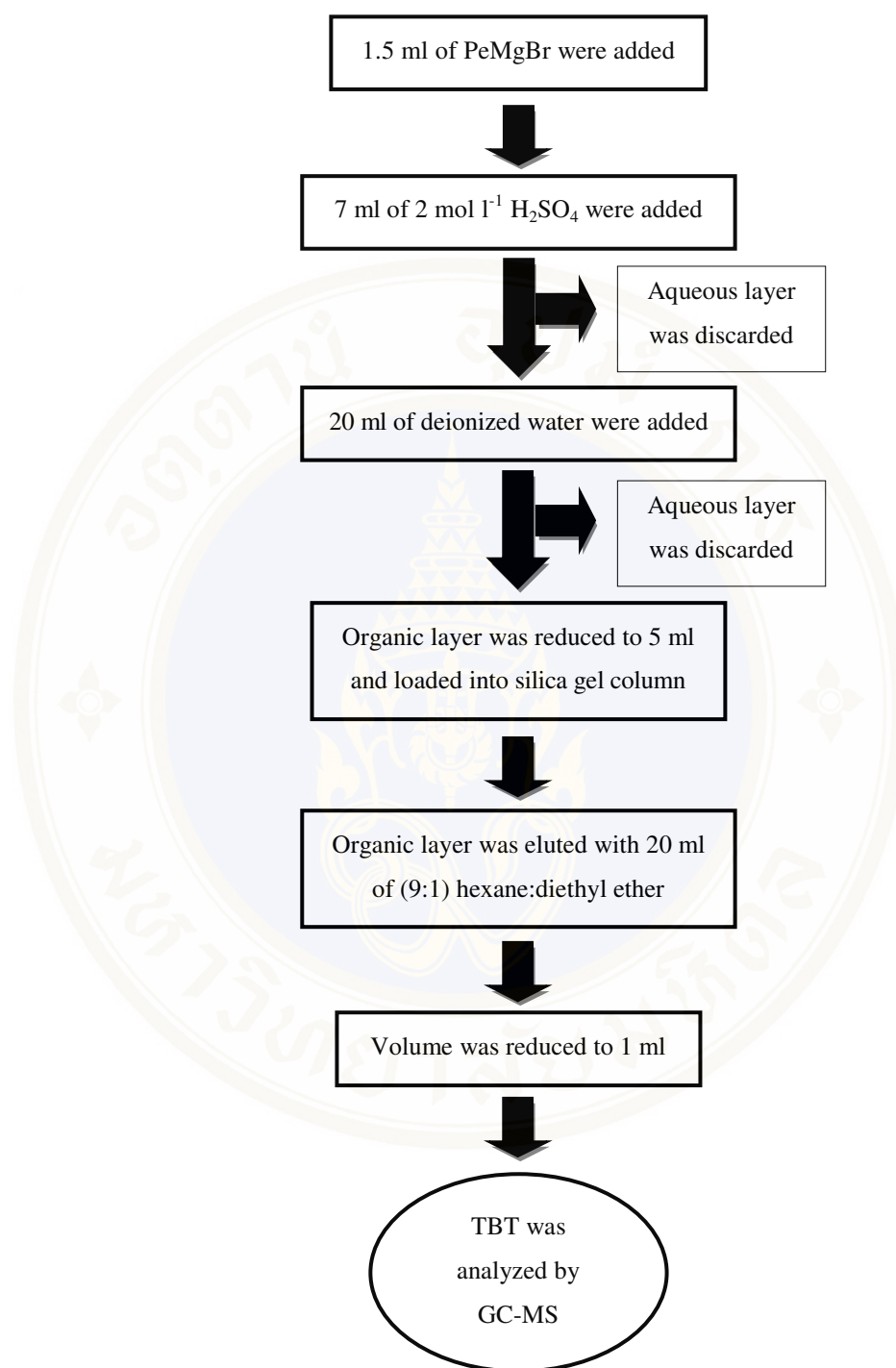
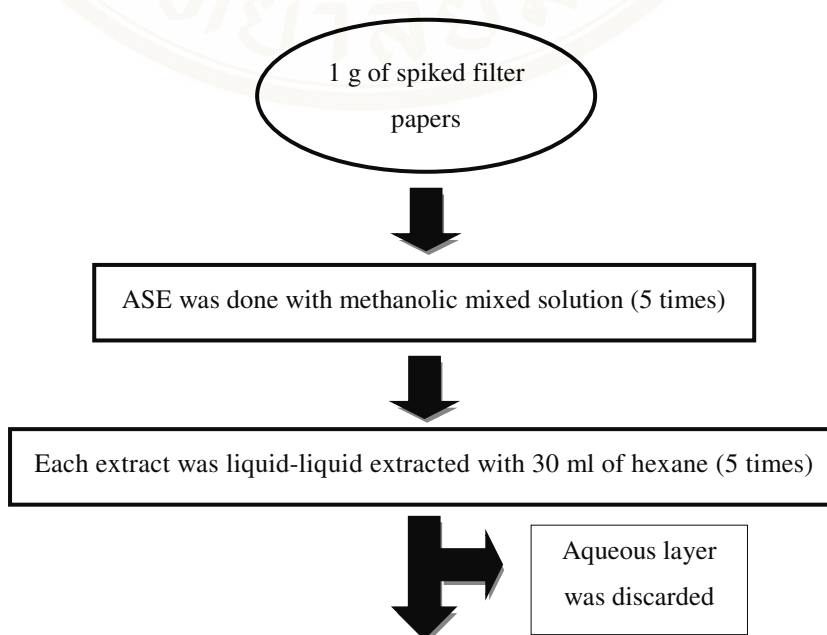


Figure 3.7 A schematic diagram of liquid-liquid extraction validation of TBT analytical procedures

3.6.1.3 Methods for biota samples

Analytical procedures of biota samples were carried out according to the flow chart of Figure 3.8. Glass filter papers were weighed 1 g and spiked with a 500 ng ml^{-1} concentration of working standard solution. The spiked filter papers were cut into small pieces and placed in an 33 ml extraction cell. The optimum ASE conditions were adopted from Wasik and Ciesielski (2004). ASE was performed and the extract was collected in the ASE vial, the extraction process was repeated for 5 times. About 35 ml of extract was transferred to 100 ml separatory funnel and liquid-liquid extracted about 10 min with 30 ml of hexane for 5 times. Subsequently all hexane layers were combined and concentrated to about 20-30 ml.

The reduced organic phase was saponificated about 1 min with 20 ml of 1 mol l^{-1} sodium hydroxide for 2 times to eliminate lipid. Then the extract was added with 20 ml of deionized water and the funnel was vigorously shaken for 1 min. After phase separation, the extract was repeatedly added with 20 ml of deionized water again. To exclude some moisture, the organic layer was added with sodium sulfate anhydrous. After that the extract was evaporated by heating with hot plate until the volume is about 10 ml and transferred to a 50 ml conical flask with rubber septum. Consequently derivatization, elution, and determination of TBT were performed same process as the validation of liquid-liquid extraction.



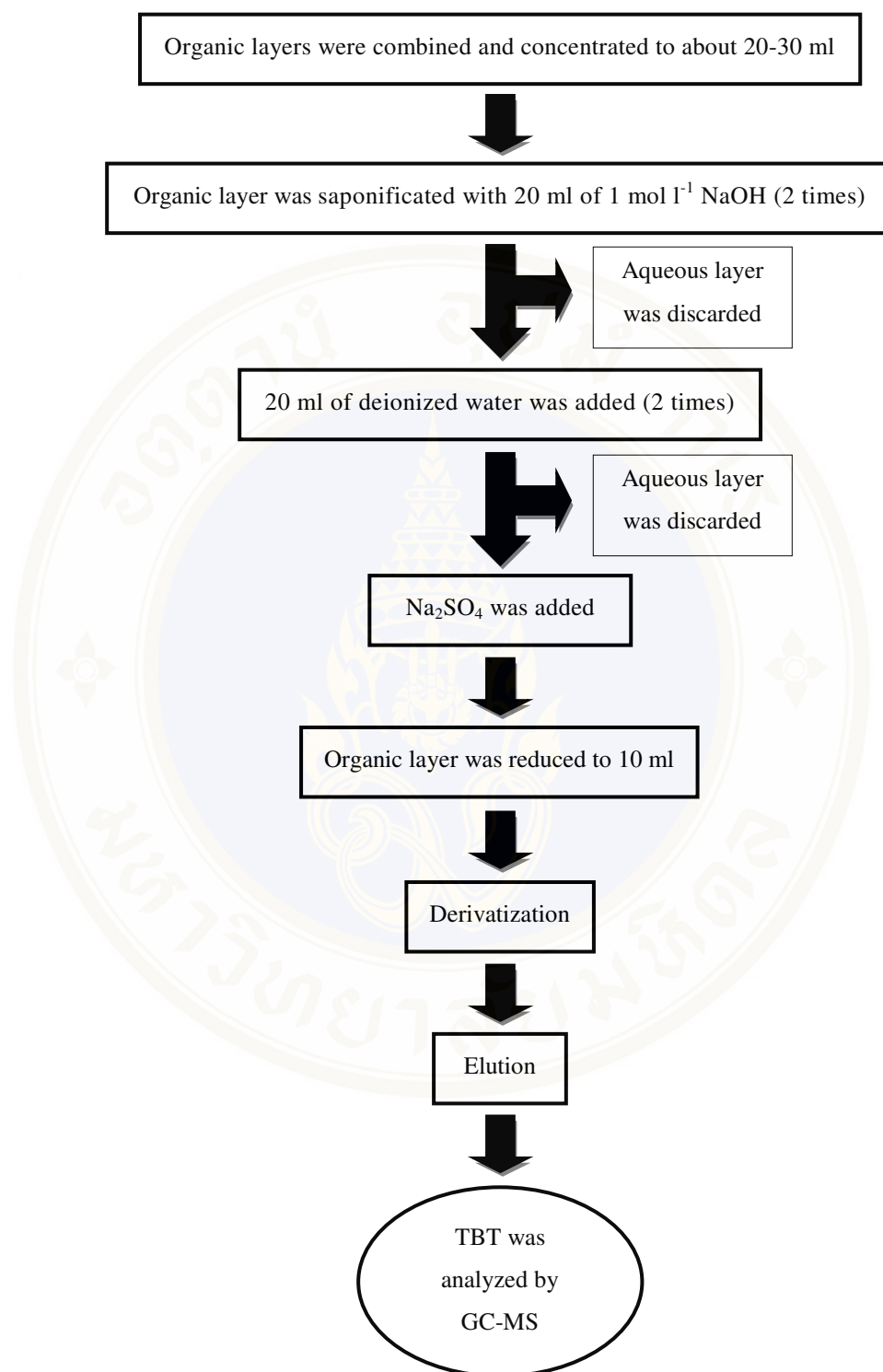
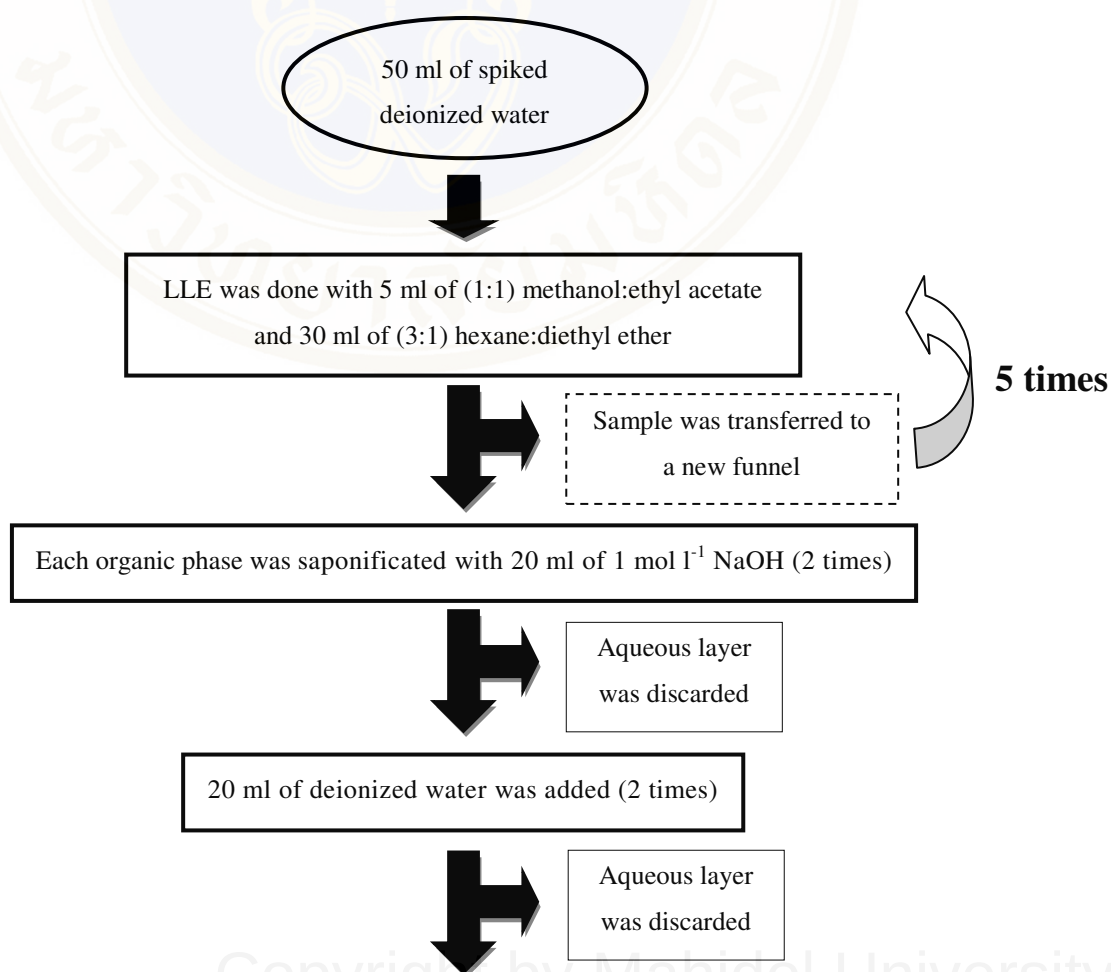


Figure 3.8 A schematic diagram of TBT analytical procedures for biota samples

3.6.1.4 Methods for water samples

About 100 ml of deionized water were acidified with 5 % hydrochloric acid (HCl, obtained from Merck) to pH 2. Then 50 ml of water were introduced to 100 ml separatory funnel and spiked with a 500 ng ml^{-1} concentration of the working standard solution. The analytical procedures are based on the method reported by Mizuishi, *et al.* (1998). The sample was added with 5 ml of (1:1) methanol:ethyl acetate (purchased from Fisher Scientific) and 30 ml of (3:1) hexane:diethyl ether. TBT was extracted from 50 ml of deionized water about 10 min for 5 times. Each organic layer was saponificated twice with the addition of 20 ml of 1 mol l^{-1} sodium hydroxide, shaken for 1 min. After the aqueous layer was released, organic layer was washed twice with 20 ml of deionized water. Then, the organic layer was added with sodium sulfate anhydrous and reduced the volume to approximately 10 ml. Derivatization, elution, and determination of TBT were performed same process as the validation of liquid-liquid extraction. A scheme was shown on Figure 3.9.



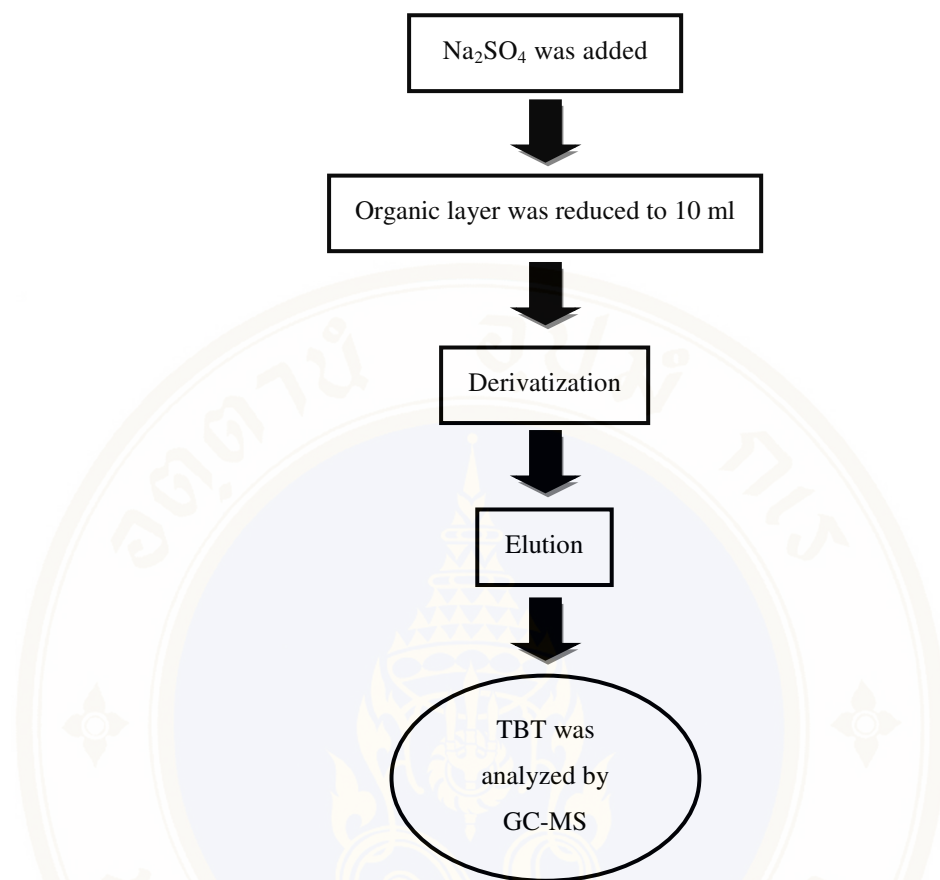


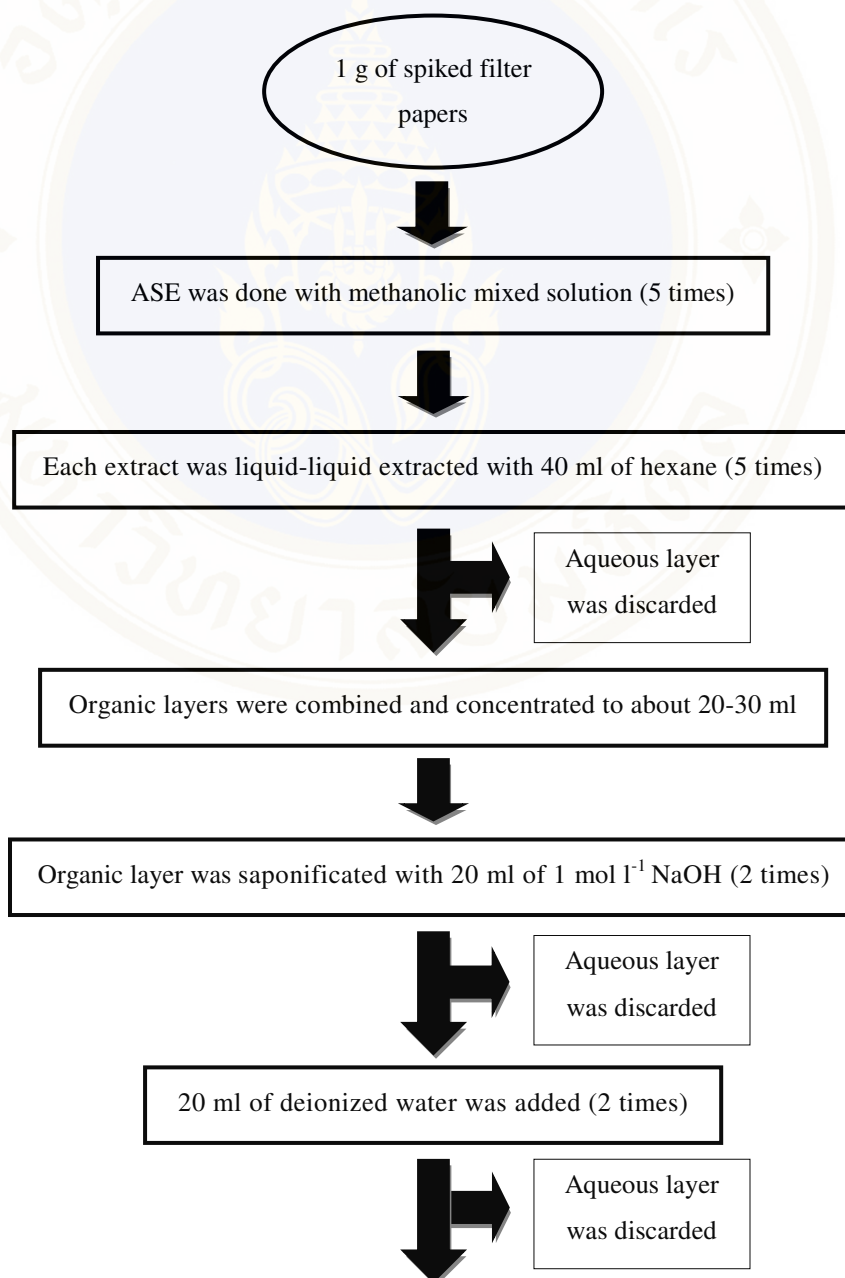
Figure 3.9 A schematic diagram of TBT analytical procedures for water samples

3.6.1.5 Methods for sediment samples

1 g of filter papers was spiked with a 500 ng ml⁻¹ concentration of the working standard solution and cut into tiny pieces before placed in an 33 ml extraction cell. The following extraction parameters were developed from Radke, *et al.* (2008): temperature 100 °C, pressure 2,000 psi, pre-heat time 5 min, heat time 5 min, static extraction time 9 min, flush volume 40 %, purge time 100 s, and 4 static extraction cycles. Solvent composition: 750 ml of methanol, 250 ml of deionized water, 1 mol of acetic acid, 1 mol of sodium acetate, and 0.6 g of tropolone. The sample was extracted and collected in the ASE vials for 5 times. About 45 ml of extract was transferred to 100 ml separatory funnel and liquid-liquid extracted with 40 ml of hexane about 10 min for 5 times.

After that all hexane layers were combined and concentrated to about 20-30 ml. The reduced organic phase was saponificated with 20 ml of

1 mol l⁻¹ sodium hydroxide about 1 min for 2 times. Simultaneously the aqueous phase was discarded and the organic layer was added with 20 ml of deionized water twice, the funnel was vigorously shaken for 1 min. To prepare the sample for derivatization step, the organic layer was added with sodium sulfate anhydrous and concentrated to approximately 10 ml. Figure 3.10 showed TBT analytical procedures for sediment samples, started with sample preparation, extraction by using accelerated solvent extractor, extraction by using liquid-liquid extraction technique, derivatization, elution, and determination of TBT were performed same process as the validation of liquid-liquid extraction.



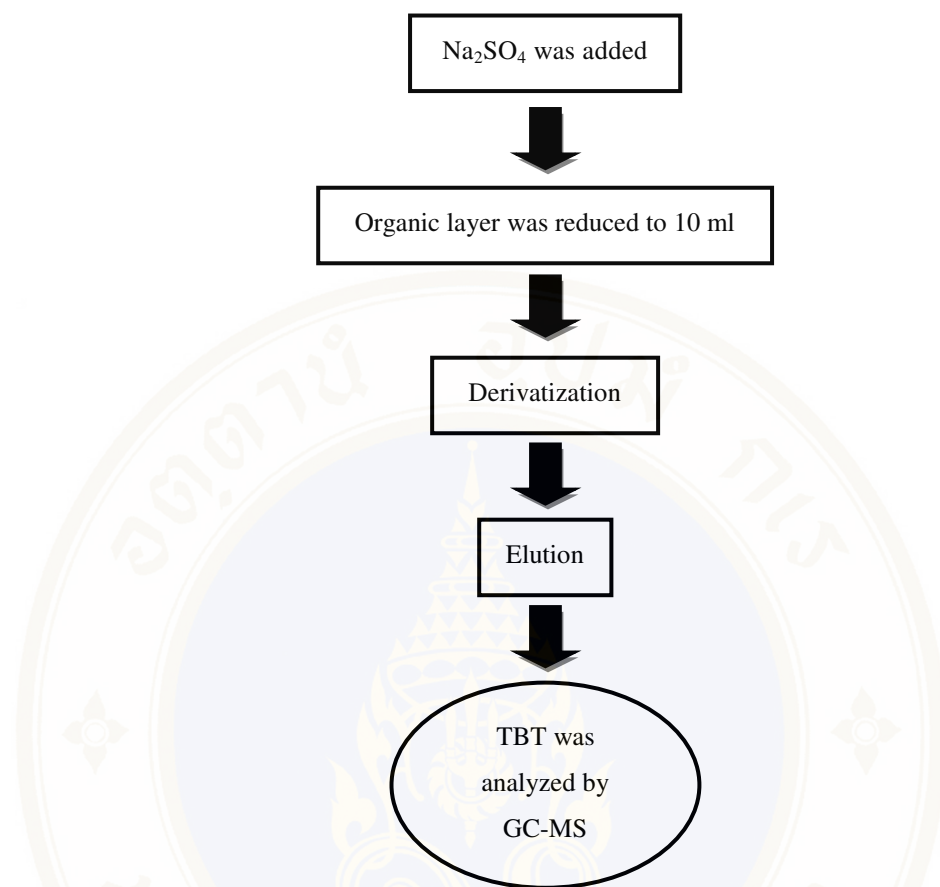


Figure 3.10 A schematic diagram of TBT analytical procedures for sediment samples

3.6.2 Field Sampling and Sample Preparation

3.6.2.1 Biota samples

Razor clam, undulated surf clam, blood cockle, and green mussel were collected from the cultural areas and placed in polyethylene ziplock bags. Samples were kept in the ice box until the analysis. Then shellfish about 100-200 g were washed with deionized water, dried in the oven at 60 °C for 24 hr, and milled into powder. The samples were kept in desiccator to avoid humidity.

3.6.2.2 Water samples

Marine water samples were collected from the aquaculture sites of shellfish by using grab method and kept in 1.5 L polyethylene bottles. Samples were

acidified to pH 2 using 5 % hydrochloric acid and stored at 4 °C on ice until the analysis.

3.6.2.3 Sediment samples

Grab method was used to take sediment samples from the surface of mud flat. Samples were placed in polyethylene containers and kept in the ice box until the analysis. Then about 100 g of sediment were dried in the oven at 105 °C for 12 hr and kept in desiccator to avoid humidity.

3.6.3 Determination of TBT in Samples

3.6.3.1 Analytical methods for shellfish

The analytical procedures were adopted from method validation. 3 g of shellfish powder were weighed and extracted for 3 times by ASE with methanolic mixed solution. About 35 ml of each extract were liquid-liquid extracted with 30 ml of hexane for 5 times. Then the hexane layers were combined and reduced to approximately 20-30 ml before saponificated with 20 ml of 1 mol l⁻¹ sodium hydroxide twice. The organic phase was added with 20 ml of deionized water for 2 times and filled with sodium sulfate anhydrous. Then all organic layers were combined and concentrated to 10 ml, and followed by derivatization step. After that pentylated organotin was evaporated with nitrogen to exactly 1 ml and loaded into a glass column with 7 g of silica gel and 1 g of sodium sulfate anhydrous. Simultaneously, sample was eluted with hexane:diethyl ether mixture and analysed by GC-MS.

3.6.3.2 Analytical methods for marine water

The analytical procedures were adopted from method validation. 50 ml of acidic marine water were placed into a separatory funnel. Liquid-liquid extraction with 30 ml of (3:1) hexane:diethyl ether in the presence of methanol and ethyl acetate was performed for 3 times. Each organic layer was saponificated twice with 20 ml of 1 mol l⁻¹ sodium hydroxide and subsequently washed twice with 20 ml of deionized water. Then the organic layer was filled with sodium sulfate

anhydrous. All organic phases were combined and concentrated to 10 ml. Consequently derivatization, elution, and determination of TBT had been done same process as method validation.

3.6.3.3 Analytical methods for sediment

The analytical procedures were adopted from method validation. Approximately 3 g of sample were weighed and extracted by ASE for 3 times with methanolic mixed solution. About 45 ml of each ASE extract were liquid-liquid extracted with 40 ml of hexane for 5 times. After that the organic layers were combined and evaporated to 20-30 ml. The reduced organic phase was saponificated with 20 ml of 1 mol l⁻¹ sodium hydroxide following by the addition of 20 ml of deionized water, and added with sodium sulfate anhydrous. Then all of the organic phases were combined and concentrated to 10 ml for derivatization reaction, subsequently elution and determination of TBT same as method validation.

3.7 Calculation of Potential Risk to Human Health

Thailand Food and Drug Administration does not provide a maximum residue limit (MRL) for TBT in shellfish or other food products. However, TBT levels in shellfish should be monitored and the potential risk to human health also should be assessed in relation to the average consumption rate in the country; in fact, the analyzed shellfish species are commonly available in fish market. The result of TBT concentration obtained from this study was used for the calculation of the human health risk associated with the consumption of contaminated shellfish. According to Penninks (1993) and EFSA (2004), the tolerable daily intake (TDI) of TBT was estimated at 0.25 µg kg body weight⁻¹ per day which is based on the observed effect of TBT on the immune function from rat to human. WHO (1999) provided the value of 0.3 µg TBT kg body weight⁻¹ per day.

Health risk assessment of TBT from shellfish exposure (expressed in µg TBT kg body weight⁻¹ per day) was calculated according to Equation 3.1 which is based on Golder associates (2007), for the estimation of adult consumer with an average

weight of 60 kg (Kannan and Falandysz, 1997; Belfroid *et al.*, 2000; Bortoli *et al.*, 2003). In this study, shellfish consumption was calculated from the average seafood consumption in Thailand adopted from FAO (1998). Mean of seafood consumption in Thailand was 71 g day^{-1} which is equivalent to $25.9 \text{ kg year}^{-1}$. The exposure scenarios were assumed that the least shellfish consumption percentage was about 5 % of a mean daily consumption of seafood and the maximum was about 100 %. The highest TBT concentration (expressed in ng g^{-1} wet weight) in four shellfish species were used for the calculation.

$$\text{Daily exposure} = \frac{\text{Consumption} \times \text{Concentration of TBT in shellfish}}{\text{Body weight}} \quad (3.1)$$

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Evaluation of The Analytical Methods

Method validation had been done in order to verify the analytical procedures employed for trial test are suitable for biological analysis. Due to lipophilic properties of TBT contribute to bioaccumulation in the high lipid contents of aquatic organisms, the biological tissues offer the advantage of being highly soluble in polar solvent. Thus, extraction by accelerated solvent extractor based on acidic digestion in the presence of a polar solvent and complexing agent was performed. After that TBT in methanolic extract was extracted with hexane by using liquid-liquid extraction technique and followed by derivatization reaction. Pentyl-magnesium bromide, Grignard reagent, was used as a derivatizing reagent which performed on only organic extract because it violently reacts with acids, water, alcohols, and ketones, etc. (Morabito and Massanisso, 2000). Therefore, liquid-liquid extraction with hexane is necessary for pentylation in order to avoid the reaction of Grignard reagent and methanolic solution. After derivatization, a portion of the hexane layer was cleaned by passage through silica gel column in order to remove any impurities before the sample was subjected to GC-MS.

A variety of analytical techniques had been developed for the speciation of organotin compounds in the different environmental components. Tutschku, *et al.* (1994) acidified the sediment samples with hydrochloric acid, then extracted them with hexane containing tropolone. Organotin compounds were derivatized with pentyl-magnesium bromide and identified by GC-AES. Milde, *et al.* (1997) ultrasonicated sediment samples with hydrochloric acid in methanol, followed by the hexane extraction containing tropolone and derivatization with methyl-magnesium iodide. Then the product was detected by GC-MS. None of TBT studies reported about extraction procedures started with accelerated solvent extraction, followed by

liquid-liquid extraction and derivatized with pentyl-magnesium bromide. Hence, the new analytical methods of this study could be the option of TBT analysis for other studies in the future.

4.1.1 Elution

Elution, also called clean up or purification step, is required for TBT analytical procedures in order to purify the derivatized target analytes in organic phase before the chromatographic separation. According to the fact that purification is necessary for biota, sediment, and soil samples, they are purified with florisil, silica gel, and alumina or ion exchange resin columns (Takeuchi *et al.*, 2000). Some studies applied sodium sulfate anhydrous in the column in order to eliminate moisture from the process. Moreover eluent, organic solvent, also plays an important role in order to carry triorganotin through the column.

From the previous studies, Regoli (1999) performed purification step by loading 1-2 ml of pentylated organotin into 5 g of alumina column. The organotin was eluted with 30 ml of (9:1) hexane:diethyl ether mixture before the determination by GC-MS. Bortoli, *et al.* (2003) reported that 0.5 ml of derivatized organotin was eluted with 10 ml of (1:1) hexane:toluene through a 3 g of activated silica gel or florisil column followed by GC-MS analysis. In addition, Wasik and Ciesielski (2004) performed the elution step by loading 1.5 ml of organic layer through Pasteur pipette filled with 1 g of alumina and a layer of sodium sulfate anhydrous on the top. The sample was eluted with 10 ml of hexane before the analysis by GC-MS.

All of previous studies reported that sample was reduced to about 0.5-2 ml which required long period of time before elution step was performed. Decrease of time consuming is very importance for TBT analysis. Therefore, method validation had been done for 3 times to optimize elution conditions for TBT purification. Hexane and diethyl ether were used to extract TBT from the column because they are the common solvent for Grignard reaction and for many other reactions involving organometallic reagents. Moreover, they are widely used as cheap, relatively safe, largely unreactive, and easily evaporated solvents.

About 10 ml of derivatized TBT standard solution (250 ng ml^{-1}) were reduced to 5 ml under nitrogen gas. Then the organic layer was loaded into the silica gel column (6 g of activated silica gel and 1 g of activated sodium sulfate anhydrous) and eluted with 10 ml of (9:1) hexane:diethyl ether mixture for 3 times, assuming that 30 ml of eluent can extract TBT from the column with 100 % efficiency. As shown in Figure 4.1, it seems likely that all 3 trials have the same trend of adsorption and desorption. TBT mean values ($n=3$) of elution steps were $0.86 \pm 0.05 \%$, $97.85 \pm 0.13 \%$, $1.20 \pm 0.12 \%$, and $0.10 \pm 0.03 \%$.

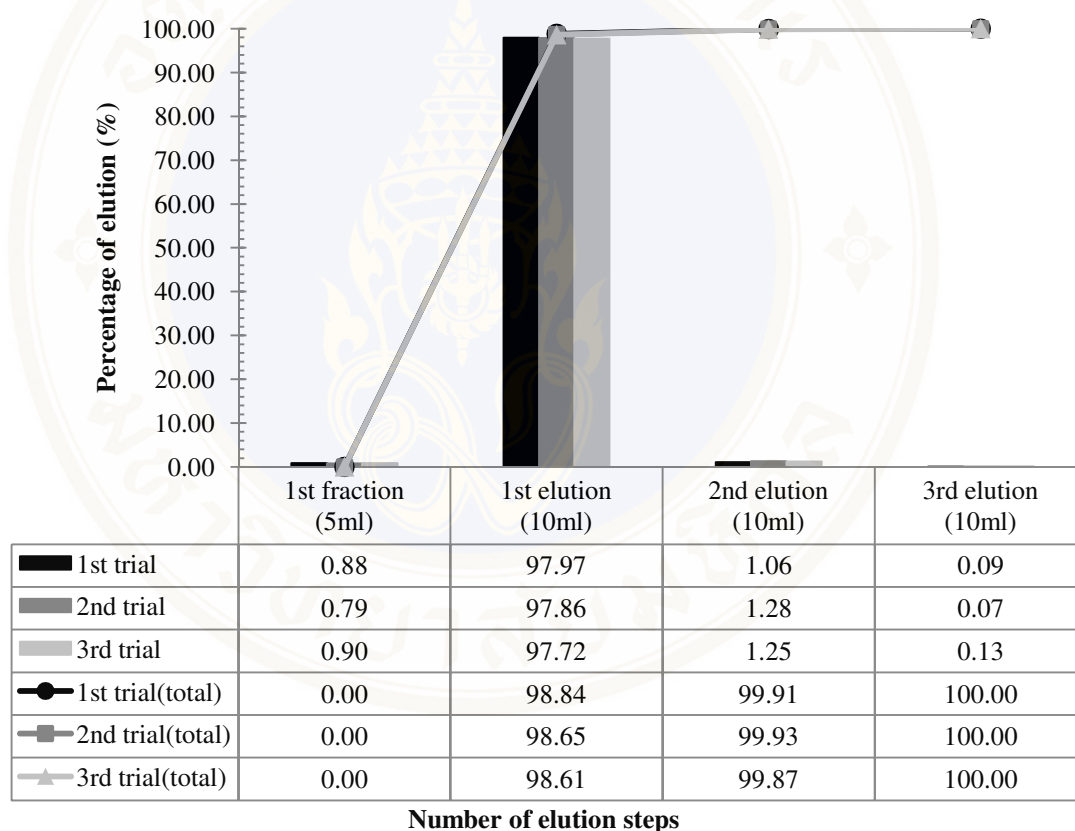


Figure 4.1 Detected TBT levels of elution steps and trend of TBT elution efficiency

Detected TBT was found in the first fraction (5 ml of sample), the highest level was found in the first elution step and it was continuously decreased until the third elution step. The least TBT level was detected in the last step, moreover, there are some co-extracted solutes and some by-products of the derivatization reaction disturbing TBT level. The average of TBT summation from

the first fraction to the second elution was about 99.90 ± 0.03 %, relative standard deviation was 0.03 % which is acceptably for this study. Therefore, the appropriate amount of eluent is 20 ml because TBT extracted from the stationary phase was detected up to almost 100 %.

4.1.2 Liquid-Liquid Extraction

There are various extraction and derivatization techniques applied for TBT analysis. Some studies reported that sample was extracted with acidic digestion in the presence of methanol and the extract was applied with Hydride generation or Ethylation by sodium tetraethylborate (NaBEt_4) in derivatization step. Others used complexing agent (Tropolone) in the presence of hexane and the extract was directly reacted with Grignard reagent. Gómez-Ariza, *et al.* (1995) mixed lyophilized bivalves with methanol containing tropolone and ultrasonicated. Then the samples were derivatized with pentyl-magnesium bromide and purified with florisil column before analysis by GC-FPD.

Nemanič, *et al.* (2002) analyzed TBT from water and mussel samples. About 1 L of unfiltered marine water was extracted twice with 30 ml of 0.3 % tropolone in dichloromethane. Mussels about 100-500 mg were sonicated with 15 ml of 0.05 % tropolone in methanol and 1 ml of concentrated hydrochloric acid. Then the samples were derivatized with 1 ml of pentyl-magnesium bromide followed by clean up with silica gel column and analyzed by GC-MS. Liscio, *et al.* (2008) extracted mussel tissue with 15 ml of (0.05 % w/v) methanol containing tropolone, then the suspension was centrifuged. The organic phase was liquid-liquid extracted with 30 ml of dichloromethane before reacted with 1.6 ml of pentyl-magnesium bromide. None of TBT studies reported about using liquid-liquid extraction with hexane extracted TBT from ASE methanolic extract, hence, it is worthy of development for a new TBT analysis.

According to alkylation reaction with Grignard reagent can only be performed in completely dry condition or non aqueous media, extraction of organic phase prior to the derivatization is necessary. Liquid-liquid extraction with organic solvent was carried out in order to extract TBT from aqueous phase before reacted with

Grignard reagent. Method validation has been done for 3 times to confirm the analytical methods. The spiked filter papers (500 ng ml^{-1} TBT) were extracted by using accelerated solvent extraction technique after that the extract was liquid-liquid extracted with hexane for 6 times in order to find the optimum number of extraction steps.

The averages of extraction efficiency from the first extraction to the sixth extraction were $45.62 \pm 1.22 \%$, $24.37 \pm 0.92 \%$, $11.17 \pm 0.17 \%$, $7.87 \pm 0.24 \%$, $6.12 \pm 0.31 \%$, and $4.76 \pm 0.37 \%$, respectively (Figure 4.2). The first liquid-liquid extraction step gave the highest TBT value and the amount of TBT was continuously decreased until the sixth liquid-liquid extraction step. It might be assumed that the average extraction efficiency of the first to the second liquid-liquid extraction with hexane can extract TBT from methanolic residue was about 50 %. In addition, the average extraction efficiency of the third to the sixth liquid-liquid extraction with hexane can extract TBT from methanolic residue was about 75 %.

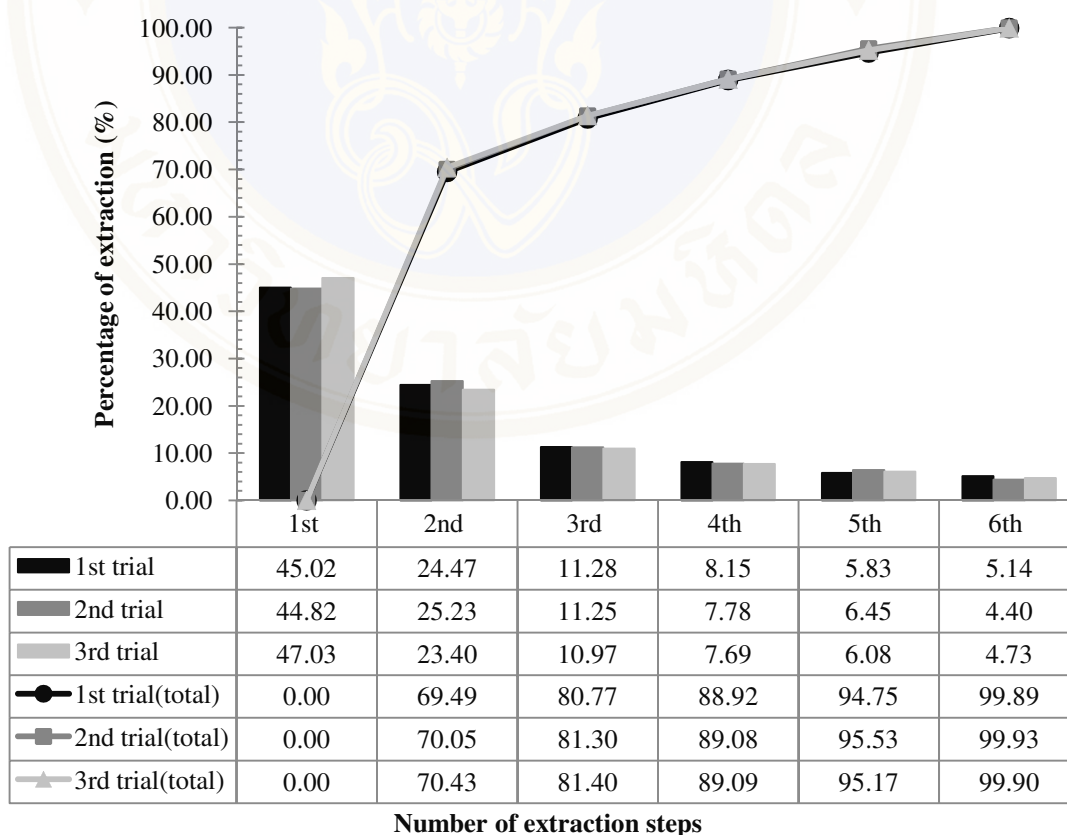


Figure 4.2 Detected TBT levels of liquid-liquid extraction and trend of liquid-liquid extraction efficiency with hexane

The average of TBT summation from the first to the sixth extraction steps was 99.91 ± 0.02 %, relative standard deviation was 0.02 %. Eventhough a higher of extraction efficiency related to the increasing of extraction step, co-extracted solutes and some by-products of the derivatization reaction were detected in the sixth extraction. If these chemicals were included in the analysis they might agitate TBT concentration. From the results, the average of TBT summation from the first to the fifth extraction steps was 95.15 ± 0.39 %, relative standard deviation was 0.41 % which is acceptably for this study. Therefore, hexane was used to extract organotin species from methanolic solution and this step could be done for 5 times.

4.1.3 Methods for Biota Samples

Accelerated solvent extraction was performed in the extraction step because it enables to control the important extraction conditions; such as solvent composition, pressure, temperature, and duration of the procedure in order to achieve more quantitative extraction. Method validation has been done with 3 trials to ensure the analytical procedures and to find out the extraction efficiency of accelerated solvent extraction. The spiked glass fiber filter papers (500 ng ml^{-1} TBT) were extracted by accelerated solvent extractor for 5 times with mixed solution followed by liquid-liquid extraction, derivatization, purification, and determination.

As shown in Figure 4.3, the first extraction step gave the highest TBT value and the amount of TBT was continuously decreased until the fifth extraction step. The averages of accelerated solvent extraction efficiency from the first round to the fifth round were 66.14 ± 5.04 %, 13.57 ± 2.39 %, 7.86 ± 4.41 %, 6.28 ± 1.91 %, and 4.96 ± 1.09 %, respectively. The result showed that all 3 trials have the same trend of extraction efficiency which gradually increases to almost a hundred percent at the fifth extraction. The average of TBT summation from the first to the fifth extraction steps was 98.81 ± 0.43 %, relative standard deviation was 0.44 %. According to the major concern about TBT analysis is time consuming, accelerated solvent extraction could be done for 3 times because it also gives a high percentage of extraction. The average of TBT summation from the first to the third extraction steps was 87.57 ± 2.52 %, relative standard deviation was 2.88 % which is acceptably for this study.

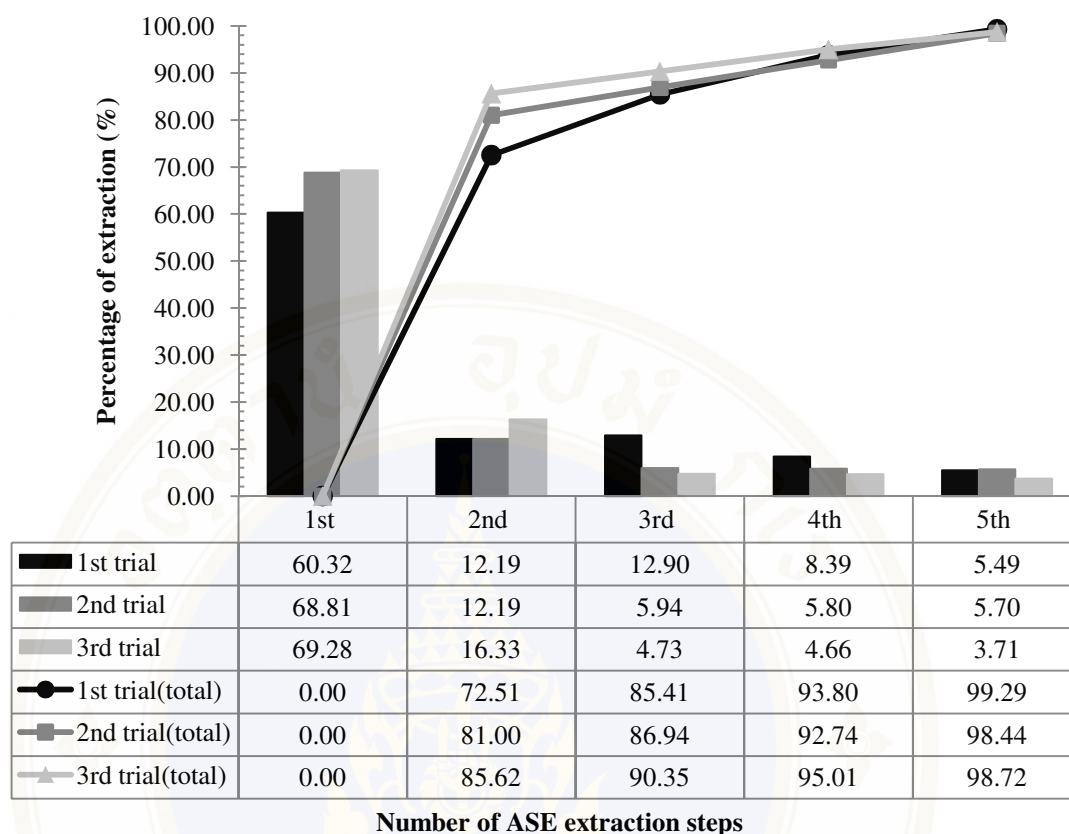


Figure 4.3 Detected TBT levels of accelerated solvent extraction and trend of accelerated solvent extraction efficiency for biota sample analysis

After that recovery test was performed, TBT was determined by spiking known amount 500 ng ml^{-1} of TBT standard solution to non-TBT glass fiber filter papers. The recovery values were obtained from 3 replicates in the range between 88-92 %. The mean value of recovery was 89.71 ± 1.70 %, relative standard deviation was 1.89 %. When compare to the results of Wasik and Ciesielski (2004) which reported that the extraction efficiency of ASE with certified reference material NIES No. 11 was 89 ± 5 %. Nemanič, *et al.* (2002) analyzed a reference material BCR CRM 477 (mussels *Mytilus edulis*), the extraction efficiency was 89 %. TBT was spiked to BCR CRM 477 for recovery test, the mean value of recovery was 118 %. It seems that the extraction efficiency and percent recovery of their study were higher than this study, it might because of the different of reference material and derivatization technique. However, this study aims to develop the new TBT analytical methods by using ASE, LLE, Grignard derivatization, and GC-MS detection which never been done for determining TBT in shellfish samples before.

Thus, these developed analytical procedures could be the option of TBT analysis for other studies in the future.

4.1.4 Methods for Water Samples

According to TBT presents in marine water at a low level, the quantification requires highly sensitive techniques or the large amount of samples with preconcentration processes. Liquid-liquid extraction, solid-phase extraction (SPE), or solid-phase microextraction (SPME) are mainly applied in extraction process. Liquid-liquid extraction was performed in the experiment because it gives the high efficiency, high enrichment factors, and reproducibility (Brunori, 2006). Method validation has been done with 3 trials to evaluate and ensure the analytical procedures. The spiked deionized water (500 ng ml^{-1} TBT) was liquid-liquid extracted for 5 times. Each extract was subsequently derivatized and followed by purification and determination.

Figure 4.4 showed that the first liquid-liquid extraction step gave the highest TBT value and the amount of TBT was continuously decreased until the fifth liquid-liquid extraction step. The averages of extraction efficiency from the first extraction to the fifth extraction were $76.28 \pm 4.53 \%$, $6.07 \pm 0.21 \%$, $4.44 \pm 1.26 \%$, $5.94 \pm 1.01 \%$, and $4.51 \pm 0.89 \%$, respectively. Trend of extraction efficiency for water sample method validation was similar in those 3 trials. The average of TBT summation from the first to fifth extraction steps was $97.25 \pm 1.90 \%$, relative standard deviation was 1.95% . The average of TBT summation from the first to the third extraction steps was $86.80 \pm 3.65 \%$, relative standard deviation was 4.20% . Due to time consuming, liquid-liquid extraction for TBT analysis in water sample could be done for 3 times because TBT value was high enough to accept for this study.

Deionized water was spiked with 500 ng ml^{-1} of TBT standard solution. Detected TBT concentration was used for recovery calculation. The recovery values (3 replicates) ranged from 89-92 %. The mean value of recovery was $90.60 \pm 1.69 \%$, relative standard deviation was 1.87% . On the other hand, Nogueira, *et al.* (2001) optimized the solid phase extraction (SPE) coupled with GC-MS for the in-house standard aqueous matrices assays. The recovery was higher than 85 % which is suitable for TBT analysis in aqueous samples. When compare with the results of

Berto, *et al.* (2007), assessed TBT in brackish waters, sediments, and the gastropods *Nassarius nitidus* in the southern Venice lagoon. The recovery of TBT in spiked brackish water samples was 97 ± 9 %. It seems likely that the recovery under this study was higher than the result of Nogueira, *et al.* (2001) but lower than the result of Berto, *et al.* (2007). In addition, the acceptance criteria of percentage recovery are about 85-115 % (Krongkrot, 2004). Thus, this study can provide a reliable result because recovery value is in the range of acceptance criteria.

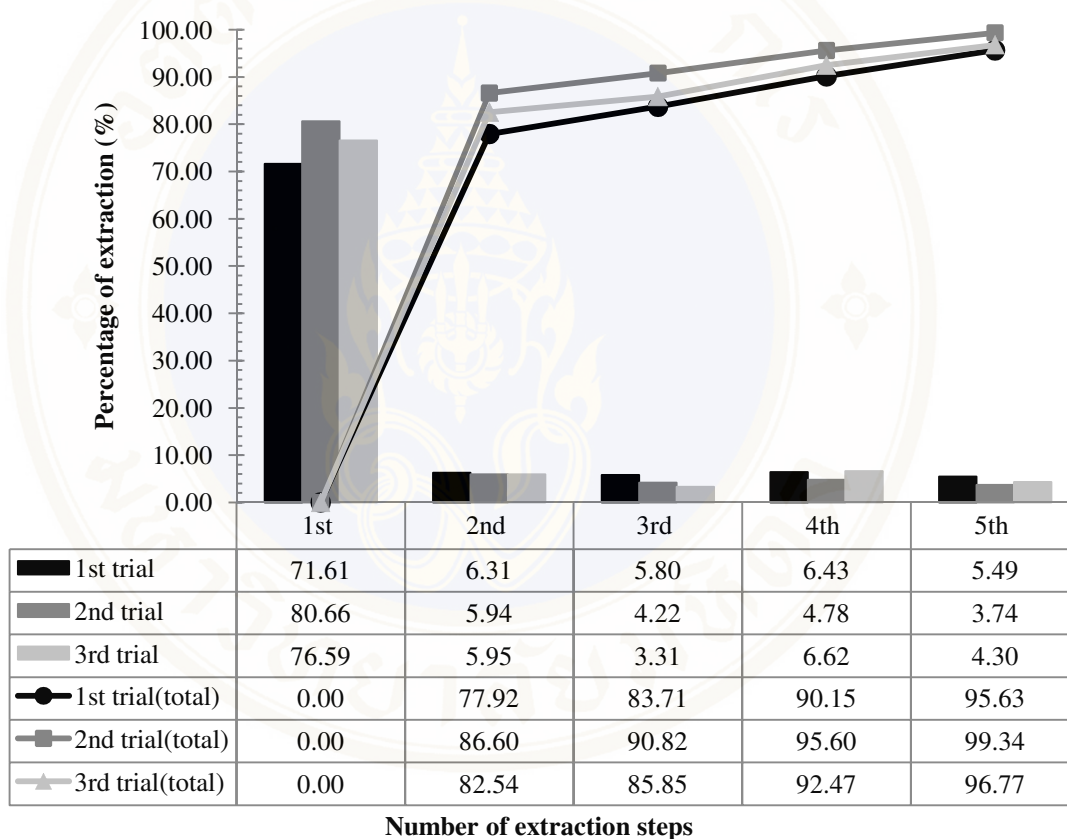


Figure 4.4 Detected TBT levels of liquid-liquid extraction and trend of liquid-liquid extraction efficiency for water sample analysis

4.1.5 Methods for Sediment Samples

In the literatures, most of methods for TBT extraction from sediment are acid, acid with polar, or acid with non polar mixtures. The application of ultrasonication, microwave extraction, and accelerated solvent extraction are widely used. In addition, the advantages of accelerated solvent extraction are the high potential of being used routinely, low solvent consumption, and the high sample throughput, etc.

(Brunori, 2006). In this study, accelerated solvent extraction was used to extract TBT from spiked glass fiber filter papers (500 ng ml^{-1} TBT) for method validation.

The highest TBT value was found in the first extraction step and the amount of TBT was continuously decreased until the fifth extraction step. The averages ($n=3$) of accelerated solvent extraction efficiency from the first round to the fifth round were $66.13 \pm 5.02 \%$, $13.14 \pm 1.80 \%$, $7.83 \pm 4.27 \%$, $6.51 \pm 2.15 \%$, and $5.14 \pm 0.88 \%$, respectively. Trend of extraction efficiency for sediment sample method validation was similar in those 3 trials. The average of TBT summation from the first to the fifth extraction steps was $98.75 \pm 1.04 \%$, relative standard deviation was 1.05% . Whereas, the average of TBT summation from the first to the third extraction steps was $87.10 \pm 2.10 \%$, relative standard deviation was 2.41% (Figure 4.5). In this case, accelerated solvent extraction could be done for 3 times because the extraction efficiency was higher than 85% which is acceptably for this study.

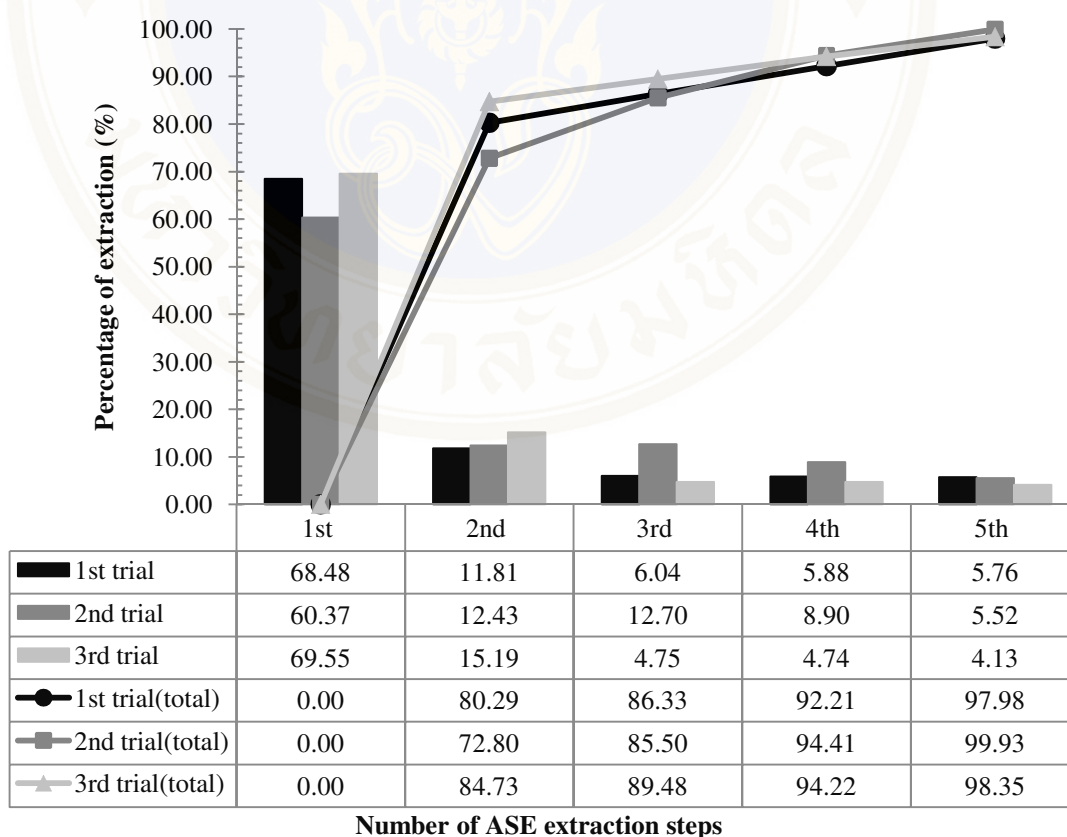


Figure 4.5 Detected TBT levels of accelerated solvent extraction and trend of accelerated solvent extraction efficiency for sediment sample analysis

Then 500 ng ml⁻¹ of TBT standard solution were added to non-TBT glass fiber filter papers for recovery test. Recoveries (3 replicates) ranged from 87-92 %. The mean value of recovery was 88.87±2.42 %, relative standard deviation was 2.72 %. From the previous studies, Berto, *et al.* (2007) reported that the recovery levels of TBT in certified reference material CRM 462 was found at 82±15 %. Conversely, Harino, *et al.* (2006) evaluated TBT analytical procedures for sediment samples by spiking 1µg of organotin standard into 5 g of sediments. Recovery of TBT in sediment was found at 107 %. From the result, the recovery under this study was higher than the result of Berto, *et al.* (2007) but lower than the result of Harino, *et al.* (2006). According to the different of reference material and analytical procedures, recoveries of those studies were difference. However, it can be assumed that TBT analytical procedures for sediment samples by using accelerated solvent extraction could be the option of TBT analysis because recovery value is in the range of acceptance criteria (85-115 %) (Krongkrot, 2004). Table 4.1 demonstrated a summary of extraction efficiency and percent recovery of analytical methods for shellfish, marine water, and sediment samples.

Table 4.1 Extraction efficiency and percent recovery of analytical methods

Type of sample	Extraction efficiency (n=3)		Percent recovery (n=3)	
	(%±SD)	(%RSD)	(%±SD)	(%RSD)
Biota	87.57±2.52	2.88	89.71±1.70	1.89
Water	86.80±3.65	4.20	90.60±1.69	1.87
Sediment	87.10±2.10	2.41	88.87±2.42	2.72

4.2 The Analysis of TBT in Marine Environment

Since method validation was done with trial samples in order to confirm the analytical methods, the determination of TBT contamination in real samples was performed. There are two parts of TBT analysis in marine samples. First, monitoring of TBT concentration in shellfish samples available in Maha Chai Market and

Don Hoi Lot Market collected in winter season. Second, determination of TBT concentration in shellfish, marine water, and sediment samples from the place of origin along coastal areas of Thailand collected in rainy season.

4.2.1 TBT Contamination in Shellfish Collected From Seafood Market

Four kinds of shellfish species were collected from the markets. Undulated surf clam, blood cockle, and green mussel were collected from Maha Chai Market, Samut Sakron Province. Whereas, razor clam, blood cockle, and green mussel were collected from Don Hoi Lot Market, Samut Songkhram Province. The measured TBT concentrations in the collected shellfish from two different locations are shown in Figure 4.6. The results showed that the highest TBT concentration was found in blood cockle (112.16 ng g^{-1} dry weight as Sn) and the lowest level was found in green mussel (26.09 ng g^{-1} dry weight as Sn) collected from Don Hoi Lot Market.

TBT concentration in blood cockles, collected from aquaculture zone which far from Don Hoi Lot seashore about 200 m, was found at 112.16 ng g^{-1} . TBT concentration in razor clams, collected at 2,500 m from seacoast, was found at 90.47 ng g^{-1} . At 3,000 m which green mussels were collected, the detected TBT concentration was found at 26.09 ng g^{-1} . It seems that two types of benthic animals, blood cockles and razor clams, have a higher TBT concentration than green mussels. Considering that Don Hoi Lot is ecotone, coastal habitats by receiving some of sediments from inland and upper watersheds (Natural Resources and Environmental Management Division, 2001). Moreover, there are many moored boats at the pier for boat tour service. This implies TBT-coated antifouling agents in ships and marine aquaculture facilities such as crab pots and fish nets accumulate in mudflats and effect on blood cockles and razor clams living on muddy soil along the coast. Whereas, the immobile mussels grew up on wood sticks in mariculture site further than the benthos, they only affected from TBT attached with particulate matter as their food sources in the water.

In addition, the levels of TBT in shellfish collected from Maha Chai Market were different. The aquaculture sites of blood cockles, green mussels and undulated surf clams are located at the coast of Phanthai Norasing Subdistrict (Samut Sakron Province). The accumulation of TBT in blood cockles collected at 500 m of

seashore was found at 64.27 ng g^{-1} . TBT concentration in green mussels collected at 2,000 m and undulated surf clams collected at 8,000 m of shoreline were found at 73.56 ng g^{-1} and 95.99 ng g^{-1} , respectively. The highest TBT concentration was found in undulated surf clams. This may be related to high fishing activities along the shipping route in the deep water which TBT might diffuse from vessels and contaminate in marine environment. According to undulated surf clams can be found in sediment far away from coastline and high water level which TBT degradation by microorganisms or ultraviolet (UV) light cannot occur easily. Thus, TBT contamination in undulated surf clams was higher than two other kinds of shellfish species.

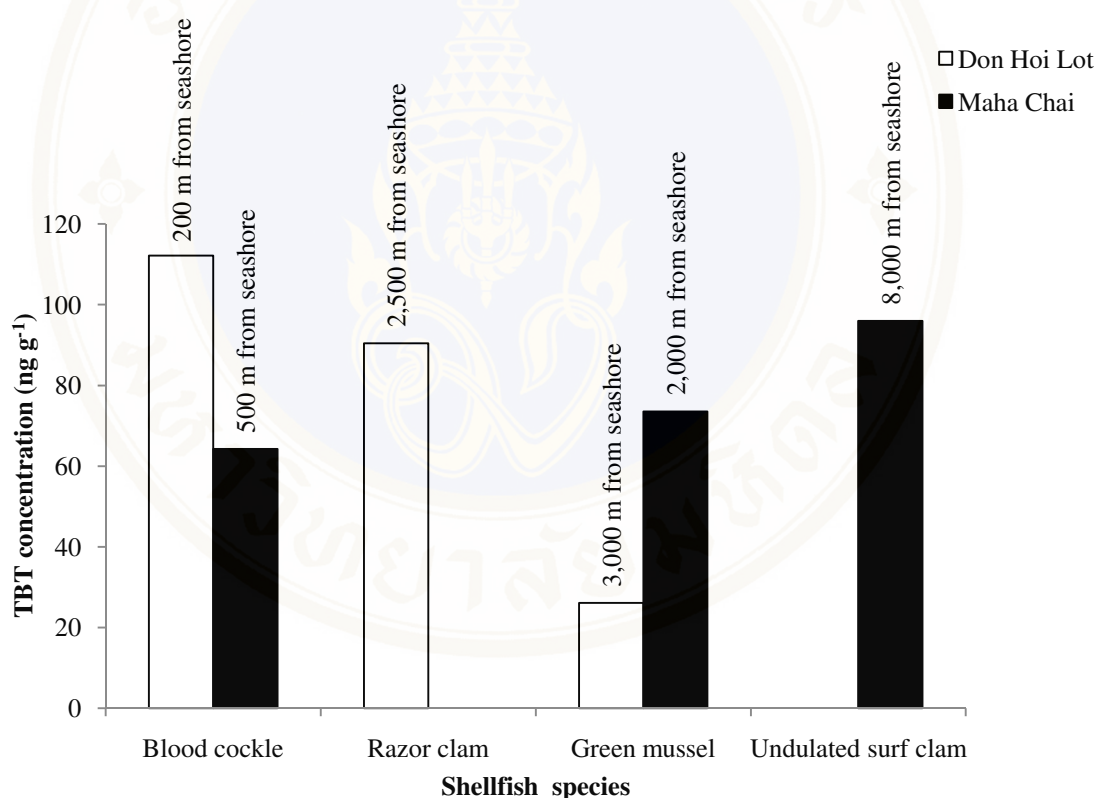


Figure 4.6 Concentrations of TBT in shellfish collected from Don Hoi Lot Market and Maha Chai Market

Blood cockles, benthos, live on muddy soil along the shore. Their food sources are the dead and decaying matter, phytoplankton, and zooplankton, etc. According to TBT concentration in sediment reflects the historical contamination (Harino *et al.*, 2006), such benthos might consume TBT in the sediment for 1-2 years

before they were taken to the market. It might be assumed that the aquaculture area of blood cockles collected from Don Hoi Lot has more pollution than the aquaculture area of blood cockles collected from Maha Chai. Nevertheless, TBT concentration in green mussels collected from Maha Chai Market was higher than TBT concentration in mussels collected from Don Hoi Lot Market. According to green mussels live on wood sticks above the seabed, their food sources are microorganisms suspended in the water. Since TBT level in green mussels reflects the concentration in water, such concentration represents the current status of chemical substance contamination in coastal water (Harino *et al.*, 2006). It might be assumed that mariculture site of mussels collected from Maha Chai has more pollution than mariculture site of mussels collected from Don Hoi Lot.

4.2.2 TBT Contamination in Shellfish, Marine Water, and Sediment Collected From Marine Aquaculture Areas

4.2.2.1 Shellfish

Four species of shellfish were taken from the different aquaculture zone along the Gulf of Thailand. TBT concentration in green mussels collected from Phanthai Norasing (Samut Sakron Province) was found at 40.92 ng g⁻¹ dry weight. Whereas TBT contamination in blood cockles from Bang Khun Sai (Phetchaburi Province), razor clams from Don Hoi Lot (Bang Ja Kreng, Samut Songkhram Province), and undulated surf clams from Mae Klong River (Samut Songkhram Province) were found at 52.17 ng g⁻¹, 62.88 ng g⁻¹, and 119.10 ng g⁻¹ dry weight, respectively (Table 4.3). The high concentrations of TBT were likely due to leaching of TBT from antifouling paint used on the hulls of the vessels. In addition, the diffusion of TBT-coated antifouling agents in marine aquaculture facilities such as crab pots and fish nets, it might accumulate in mudflats and effect on living organisms.

The levels of concentrations in the different shellfish species depend on their mobility, habitat, and behavior. Green mussels, the coastal bivalves, were grown on sticks above the seabed, they were fed naturally by plankton in the water. As the previous description of TBT characteristic, it highly adsorbs on particles and precipitates to the sediment layer but some part in the water can be degraded by UV radiation. Green mussels might consume some TBT which is not

photolyzed yet, thus, TBT can accumulate in the organs which containing high level of lipid in their bodies. On the other hand, the benthos such as blood cockles, razor clams, and undulated surf clams lives on the sediment surface layer were found higher TBT concentration than green mussels. Especially, the highest TBT concentration was found in undulated surf clams which living in sediment far away from coastline and high water level. They might up take the accumulated TBT in the sediment which is their habitation.

Till now, a few studies have reported the contamination of TBT in shellfish samples, nevertheless, there are some papers investigated TBT in green mussels in the Asia-Pacific region. Kan-atireklap, *et al.* (1997a) measured butyltin (BT) residues in green mussels collected in 1994 and 1995 from coastal waters of Thailand. TBT concentration ranges were from 7-200 ng g⁻¹ wet weight (mean=64 ng g⁻¹) in 1994 and 3-680 ng g⁻¹ wet weight (mean=90 ng g⁻¹) in 1995. Based on the study of Page and Widdows (1991), the degrees of TBT concentration in mussel tissues were rated as “low” to “moderate”, except for a location in Kung Kra Baen, Chanthaburi Province, with “heavy” TBT contamination (680 ng g⁻¹). Tanabe, *et al.* (2000) reported the contamination of butyltin (BTs) and organochlorine compounds (OCs) in green mussels collected along the coastal areas of Thailand, Philippines and India during the period of 1994-1997 were in the ranges of 3-680 ng g⁻¹, <1-640 ng g⁻¹, and <1-150 ng g⁻¹ wet weight, respectively.

Bech (2002) investigated how tributyltin contamination resulting from the establishment of a new marina and increasing yachting activities at Phuket Island, Thailand. Sudaryanto, *et al.* (2002) surveyed TBT concentration in mussels from Asian developing countries in 1997-1999. Mean TBT level in mussels from Cambodia, China, India, Indonesia, Malaysia, the Philippines, and Vietnam were 2.38 ng g⁻¹, 26.1 ng g⁻¹, 11.9 ng g⁻¹, 2.14 ng g⁻¹, 18.3 ng g⁻¹, 1.95 ng g⁻¹, and 2.61 ng g⁻¹ wet weight, respectively. In the late 20th century, the concentrations of TBT in green mussels collected from Thailand coastal were higher than other developing countries in the Asian region as shown in Table 4.2. These results could indicate that TBT-based antifouling paints were widely used in Thailand due to a rapid increase of industrial activities and high aquaculture activities (Hungspreugs *et al.*, 1989).

Table 4.2 Concentrations of TBT in green mussels collected from Asian developing countries

Location	Concentration (ng g ⁻¹ wet weight)	References
Thailand, 1994-1997	81.00	Tanabe <i>et al.</i> , 2000
The Philippines, 1994-1997	85.85	Tanabe <i>et al.</i> , 2000
India, 1994-1997	17.16	Tanabe <i>et al.</i> , 2000
Cambodia, 1997-1999	2.38	Sudaryanto <i>et al.</i> , 2002
China, 1997-1999	26.1	Sudaryanto <i>et al.</i> , 2002
India, 1997-1999	11.9	Sudaryanto <i>et al.</i> , 2002
Indonesia, 1997-1999	2.14	Sudaryanto <i>et al.</i> , 2002
Malaysia, 1997-1999	18.3	Sudaryanto <i>et al.</i> , 2002
The Philippines, 1997-1999	1.95	Sudaryanto <i>et al.</i> , 2002
Vietnam, 1997-1999	2.61	Sudaryanto <i>et al.</i> , 2002

However, TBT level in mussels collected from Thailand declined from the past (Figure 4.7). Lommetta (2001) reported that TBT contamination in green mussels collected from mariculture sites in Chonburi Province was 24 ng g⁻¹ wet weight. Harino, *et al.* (2006) measured TBT in green mussels from Thailand, the mean concentration of TBT was 16.2 ng g⁻¹ wet weight. From the results under this study in 2008, TBT concentration in green mussels collected from Maha Chai Market was found at 73.56 ng g⁻¹ dry weight (equivalent to 5.98 ng g⁻¹ wet weight) and Don Hoi Lot Market was found at 26.09 ng g⁻¹ dry weight (equivalent to 2.37 ng g⁻¹ wet weight). The average TBT concentration in mussels was 4.18 ng g⁻¹ wet weight. In addition, TBT level in green mussels collected from the farm at Phanthai Norasing Subdistrict, Samut Sakron Province in 2010 was found at 3.85 ng g⁻¹ wet weight (equivalent to 40.92 ng g⁻¹ dry weight). According to Harino, *et al.* (2003), water contents of mussels are about 80-90 %.

As a result, it can be assumed that the usage of TBT-coated antifouling paints decreased from 15 years ago.

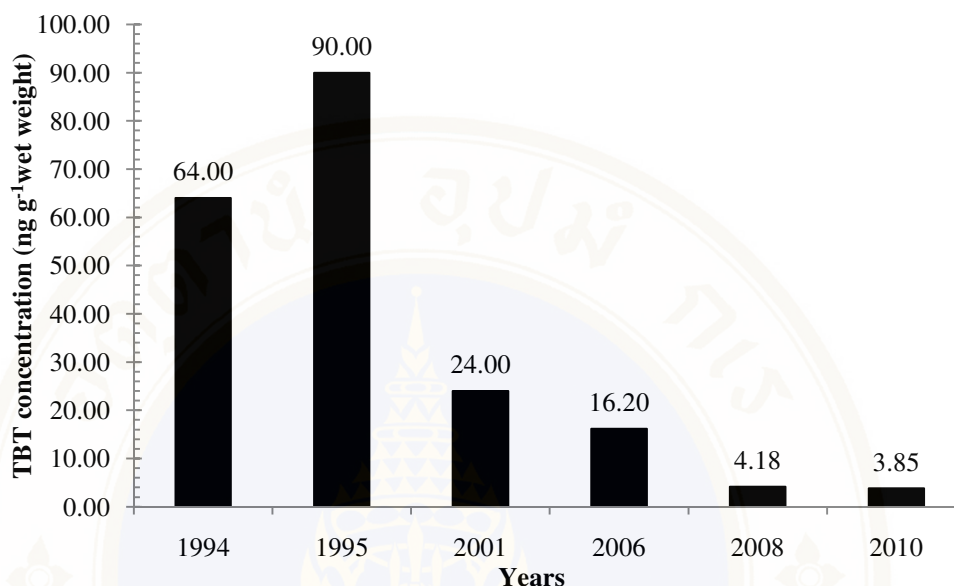


Figure 4.7 The concentrations of TBT in green mussels collected from Thailand during 1994-2010

Figure 4.8 showed the concentrations of TBT in shellfish collected in 2008 and 2010 under this study. Green mussels were collected in November, 2008 and May, 2010 from the same aquaculture place (Phanthai Norasing, Samut Sakron Province). TBT concentration in mussels collected in 2010 (40.92 ng g^{-1}) was lower than in 2008 (73.56 ng g^{-1}). TBT concentrations in blood cockles collected from Don Hoi Lot Market and Maha Chai Market in 2008 were 112.16 ng g^{-1} and 64.27 ng g^{-1} , mean TBT concentration was 88.22 ng g^{-1} . Whereas, TBT accumulation in cockles collected from Bang Khun Sai, Phetchaburi Province (The largest nature area of cockle in Thailand) in 2010 was 52.17 ng g^{-1} . Likewise, TBT values in razor clams collected from Don Hoi Lot in 2008 and 2010 were 90.47 ng g^{-1} and 62.88 ng g^{-1} . According to life cycle of shellfish is about 1-2 years, growth rate of those species is influenced by the availability of food, temperature, salinity, and water movement during a year.

International Maritime Organization (IMO) stated that on 1st January 2008 is the last date for having a TBT-based antifouling paints on a vessel. Further, PCD (2008) reported that in 2006 most of antifouling paint production in

Thailand already stopped using TBT. Therefore, TBT concentrations in green mussels, blood cockles, and razor clams collected in 2010 were lower than TBT concentrations in those shellfish species collected in 2008. However, there were some antifouling paints which contain TBT still available in the market and illegal imported. It might be because TBT antifouling paints have a lower cost than non-TBT antifouling paints. This implies that TBT-coated antifouling paints are still being used for ships, vessels, and marine equipments. Moreover, the illegal discharge of wastewater which contains TBT from the cooling pipe of the industries might spread in marine water. Such toxic chemical continuously accumulates in the sediment through the year pass by and effects to an aquatic organisms living in marine environment.

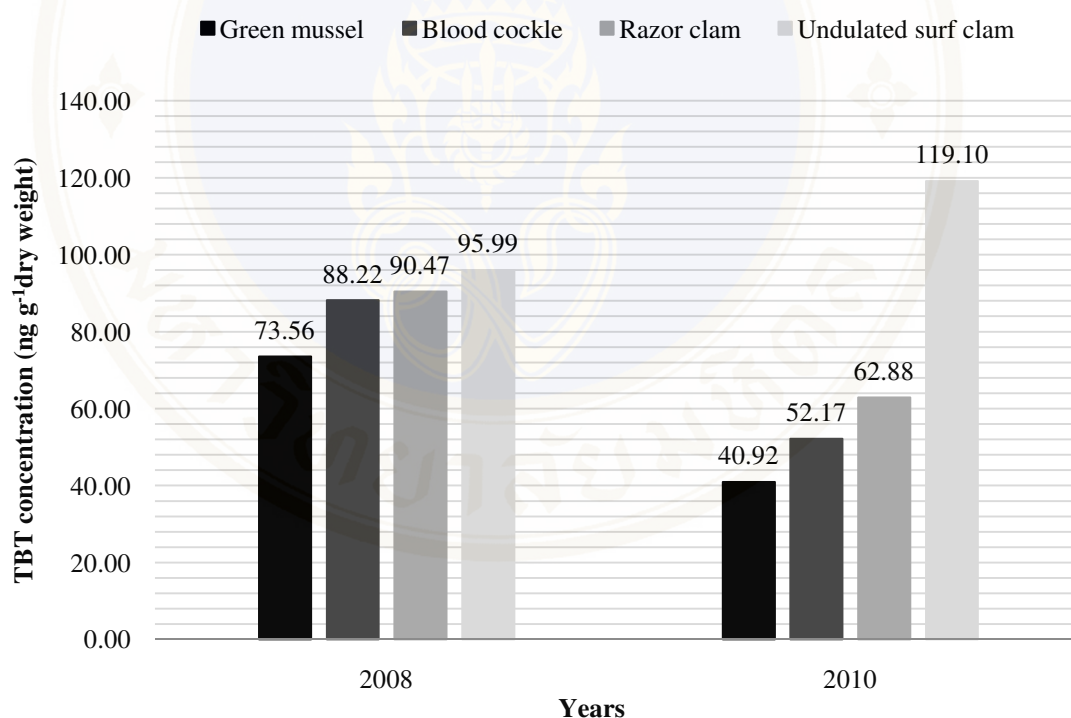


Figure 4.8 The concentrations of TBT in shellfish in 2008 and 2010

From the results under this study, TBT concentrations in green mussels, blood cockles, and razor clams collected in 2010 reduced from 2 years ago. Conversely, TBT concentration in undulated surf clams (119.10 ng g^{-1}) collected from Mae Klong River, Samut Songkhram Province in 2010 was higher than TBT contamination in undulated surf clams (95.99 ng g^{-1}) collected from Phanthai

Norasing Subdistrict in 2008. Due to undulated surf clams bury themselves about 20 cm in mudflat below water surface around 6-8 m, life cycle of such shellfish species is 1-2 years. The main food sources for the benthos are algae, plankton, and organic runoff from land. When TBT diffuses from coastal aquaculture facilities and vessels mooring in the harbors or leaches from industrial wastewater along Mae Klong River, it might attach and accumulate in those food sources where the light does not penetrate to the seabed. Therefore, undulated surf clams might consume undegraded TBT while they were feeding. From the result, it might be assumed that the aquaculture area of undulated surf clams in Mae Klong River is more polluted than the mariculture site of Phanthai Norasing Subdistrict.

4.2.2.2 Marine water

Table 4.3 gives the results of four water samples collected from the different aquaculture areas of shellfish in 2010. The least polluted marine water sample was found at 0.90 ng ml⁻¹ of blood cockles' aquaculture area. While TBT in water samples taken from green mussels' aquaculture area, razor clams' aquaculture area, and undulated surf clams' aquaculture area were found at 1.15 ng ml⁻¹, 1.19 ng ml⁻¹, and 1.30 ng ml⁻¹, respectively. From the result, it seems that the depth of the water and boating activities around the cultural areas have correlation with TBT concentration in water samples. The natural mariculture of blood cockles and razor clams is the shallow tidal flat where the water exposes to the sunlight, photolysis can perform easily. Therefore, detected TBT in water sample taken from mariculture of blood cockles was less than marine water from other places. Conversely, TBT level in marine water from mariculture of razor clams was high. It can be assumed that leaching of TBT from the different density of fishing activity effects on TBT contamination in marine water.

The highest TBT concentration was found in water sample collected from the aquaculture area of undulated surf clams. Sources of TBT might come from high boating activities along Mae Klong River or wastewater discharged from the industries. TBT degradation in the high water level of polluted river cannot occur because UV degradation simply takes place in situation where TBT in the water

is exposed to sunlight. PCD (2010) evaluated surface water quality of Mae Klong River in 2010, the classification of water quality is more inferior (Class 4; fairly clean fresh surface water resources) than the previous classification in 1994 which specified in Class 3 (medium clean fresh surface water resources). The report was revealed that the water is dirtier than it used to be in the last 16 years, moreover, organic matter was found in range 0.7-2.3 mg l⁻¹ which is higher than standard.

According to WHO (1990), TBT degradation rates in water are faster than in sediment. Half-lives of TBT in marine water are 4-60 days, however, those in sediment have been found about 1-15 years (Omae, 2006). Thus, TBT contamination in marine water was lower than two other kinds of samples. Although sunlight and microorganisms play an important role in TBT degradation, there have been some studies revealed about the rate of degradation taken place in the dark. Focardi, *et al.* (2000) reported that the sorption of TBT to the sediment was rapid, the equilibration of TBT between particulates and water was started up within 10 min and achieved within 2 hr. They found that 85.7 % of the added TBT had bound with suspended solids in the dark at 15 °C. Moreover, data for the half-lives of TBT in natural marine water, in fresh water, in estuarine water, or in pure water are difference according to the intensity of light, temperature, flow rates of marine water, salinity, and types or amounts of organic matter (Omae, 2006).

Lommetta (2001) reported TBT contamination in coastal water along the eastern part of the upper Gulf of Thailand in 2000. There are six fishing boat piers, three coastal aquaculture areas, and two commercial vessel harbors. TBT concentrations in the water samples ranged from 0.069-0.223 ng ml⁻¹, 0.069-0.146 ng ml⁻¹, and 0.113-0.144 ng ml⁻¹, respectively. Pollution Control Department (PCD, 2003; 2004; 2005) reported that the concentrations of TBT in coastal water of Thailand (23 coastal provinces) ranged from <0.002-0.007 ng ml⁻¹ in fishing areas with small boats and aquaculture facilities. Whereas in high fishing activities near large harbors and major shipping areas ranged from 0.012-0.140 ng ml⁻¹. From the result, it showed that TBT concentrations in marine water under this study in 2010 were higher than the results of those previous studies and Thailand Coastal Water Quality Standard for TBT (0.010 ng ml⁻¹).

4.2.2.3 Sediment

TBT concentrations at all the sampling locations were reported in Table 4.3. The least polluted sediment sample was found at 104.34 ng g⁻¹ dry weight of green mussels' aquaculture area. Whereas TBT contamination in sediment samples from blood cockles' aquaculture area, razor clams' aquaculture area, and undulated surf clams' aquaculture area were found at 151.94 ng g⁻¹, 165.41 ng g⁻¹, and 212.53 ng g⁻¹ dry weight, respectively. The highest TBT contamination in sediment was found in the aquaculture area of undulated surf clams. Such benthos lives in marine sedimentary environment below the surface about 6-8 m. As the prior mentions, there are high boating activities along Mae Klong River, hence, TBT might leach from the vessels and accumulate in sediment. According to TBT easily forms with the oxygen atoms of metal oxides in sediment or nitrogen atoms of proteins in those fish and shellfish (Omae, 2006). Moreover, particular concern is the long persistence of TBT in sediment where photolysis cannot take place. Sediment might be a big reservoir for TBT in some location. Therefore, TBT accumulation in sediment sample taken from seabed was higher than other samples.

The analysis of TBT contamination in sediments from coastal areas of Thailand in 1995 was conducted by Kan-atireklap, *et al.* (1997b). TBT concentrations ranged from 4-81 ng g⁻¹ at coastal aquaculture sites, 7-93 ng g⁻¹ at fishing boat piers/coastal aquaculture sites, 9-880 ng g⁻¹ at fishing boat piers/ports, and 36-4,500 ng g⁻¹ dry weight at offshore vessel harbors. The high TBT levels were considered to be related with large commercial shipping or far seas vessels rather than high boating activities or coastal aquaculture areas. Lommetta (2001) reported that TBT levels in surface sediments at fishing boat piers, coastal aquaculture sites, and commercial vessel mooring harbors ranged from 2-11 ng g⁻¹, 27-87 ng g⁻¹, and 11-31 ng g⁻¹ dry weight, respectively.

Harino, *et al.* (2006) monitored TBT in sediments around the inner Gulf of Thailand. TBT concentrations were found in range 2-1,246 ng g⁻¹ dry weight. The highest TBT concentration was found at shipyard area with repair facilities, the deposit of TBT antifouling paints to sediment might occur. However, TBT in sediments collected from the aquaculture areas ranged from 3-34 ng g⁻¹ dry weight. Especially, TBT concentration in sediment collected from Mae Klong River was found

at 10 ng g^{-1} dry weight. When compare the results of this study with the results of the earlier studies, TBT levels in sediments collected from mariculture sites under this study were higher than those previous studies. It can be assumed that TBT is still being used as an antifouling paint for aquatic facilities and boat-bottom paints. TBT might leach from those sources and continuously accumulated in sediment where degradation cannot occur easily.

Table 4.3 TBT concentrations in shellfish, marine water, and sediment samples from various locations

Shellfish species	Sampling location	Concentration		
		Shellfish	Water	Sediment
		(n=3) ($\text{ng g}^{-1} \pm \text{SD}$)	(n=3) ($\text{ng ml}^{-1} \pm \text{SD}$)	(n=3) ($\text{ng g}^{-1} \pm \text{SD}$)
Green mussels	Phanthai Norasing Subdistrict, Samut Sakron Province	40.92 $\pm$ 1.67	1.15 $\pm$ 0.06	104.34 $\pm$ 1.57
Blood cockles	Bang Khun Sai Subdistrict, Phetchaburi Province	52.17 $\pm$ 0.98	0.90 $\pm$ 0.02	151.94 $\pm$ 1.44
Razor clams	Bang Ja Kreng Subdistrict, Samut Songkhram Province	62.88 $\pm$ 1.89	1.19 $\pm$ 0.04	165.41 $\pm$ 5.78
Undulated surf clams	Mae Klong River, Samut Songkhram Province	119.10 $\pm$ 2.83	1.30 $\pm$ 0.09	212.53 $\pm$ 11.91

The majority of TBT measurements in green mussels were generally below from the past, besides the analysis in blood cockles and razor clams also showed the results in the same way. However, TBT accumulation in undulated surf clams was found higher than the prior analysis. According to food, environment, and habitation affect on the development of shellfish. Although, the prohibition of the use of TBT paints on vessels shorter than 25 m in length in the USA, Australia, Japan, and in many EU countries was effective. TBT is still being used in antifouling paints applied on vessels larger 25 m in length in most developed countries and developing countries, including Thailand (Wattayakorn, 2008). Therefore, TBT can still be encountered in the environment. As in marine water and sediment cases, the

concentrations of TBT in those samples collected from mariculture of shellfish along the inner Gulf of Thailand were shown to be increased as compared with researches from the past.

4.2.3 Interaction of TBT Contamination in Shellfish, Marine Water, and Sediment

Shellfish, marine water, and sediment were collected from the aquaculture sites along the coastal northernmost part of the Gulf of Thailand in order to estimate TBT concentrations and study the correlation of TBT accumulation in those mediums. From the result, the lowest TBT concentration was found in marine water while TBT contamination in shellfish was found higher, and the highest level was found in sediment (Figure 4.9). As the prior mentions, the study areas of this research are aquaculture sites of shellfish, it can be assumed that most of TBT sources affect to marine environment might come from fishing activities which TBT antifouling paints were applied on small boat and aquatic facilities (Grovhoug *et al.*, 1986). When TBT enters the water, such chemical easily forms complex with oxygen atoms in metal oxides of the sediment. Many studies explained that TBT adsorption depends on the different factors, for examples, turbidity, salinity, water movement, and temperature, etc. (WHO, 1990). Once the complex of TBT precipitates in sediment, it takes a long period of time to be degraded, thus the highest TBT concentration was found in sediment samples. While TBT in the water might be photolyzed by sunlight, hence, TBT in marine water was found at the lower level than TBT in sediment.

Omae (2006) reviewed the study of TBT degradation in marine environment. The evidence showed that the microorganisms and sunlight play an important role in the degradation of TBT. Moreover, TBT degradation rates were high in light when compared with those of degradations in the dark. The half-lives of TBT in marine water ranged from 4-60 days, whereas, those in sediment have been reported to be about 1-15 years. de Mora, *et al.* (1995) investigated degradation rate of TBT in sediment cores collected from Tamaki Estuary in Auckland, New Zealand in 1989. A plot of the natural logarithm of concentration against time, the average degradation rate was 37.5 % per year and a half-live of 1.85 years. Degradation of TBT by

microorganisms in water and sediment from the harbor area of Osaka City in Japan was studied by Harino, *et al.* (1997).

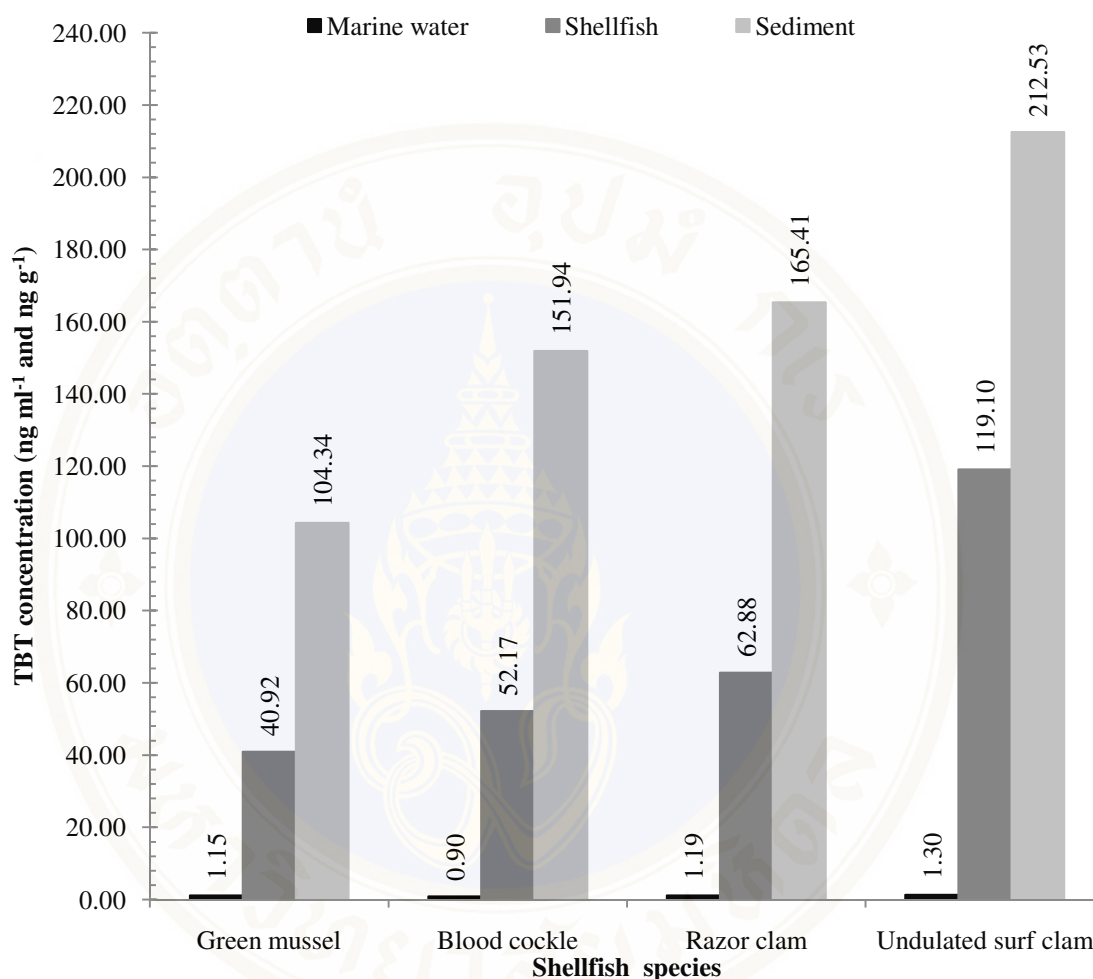


Figure 4.9 The concentrations of TBT in shellfish, marine water, and sediment

From the result under this study, TBT contamination in shellfish, marine water, and sediment were found in the range from 40.92-119.10 ng g⁻¹ dry weight, 0.90-1.30 ng ml⁻¹, and 104.34-212.53 ng g⁻¹ dry weight, respectively. According to microorganisms in water degraded TBT rather faster than those from sediment, TBT level in water was lower than TBT level in sediment. In addition, shellfish living in marine ecosystem were influenced by TBT adsorbed in phytoplankton or contaminated sediment. The classification of shellfish in this study can be divided into two types. Green mussels, ciliary-mucus suspension feeders, the immobile mussels attached to rocks or wood sticks and fed by particulate matter suspended

in the water. Benthos is an aquatic animal lives on the seafloor such as, blood cockles, razor clams, and undulated surf clams.

Food sources for green mussels are the dead and decaying matter, phytoplankton, zooplankton, dinoflagellate, flagellate, and protozoa suspended in marine water. Therefore, TBT attached with those organisms has an influence on the growth of green mussels. Because TBT concentrations in mussels reflect the concentrations in water, those concentrations represent the current status of such chemical substance contamination in coastal water. While TBT continuously accumulates in the sediment, the levels of TBT in sediment reflect the historical contamination. Benthic animals are the sediment eaters. Their food sources are detritus, phytoplankton, zooplankton, microalgae, and diatom. TBT might accumulate in such benthos through the food chain.

TBT value in blood cockles was about 34 % of TBT value in sediment from the aquaculture site. In addition, TBT value in razor clams and undulated surf clam were about 38 % and 56 % of TBT value in sediment. The results showed that TBT level in undulated surf clams was found more than 50 % of TBT level in sediment. However, TBT levels in blood cockles and razor clams were found lower than 50 % of TBT level in sediment. According to the fact that blood cockles and razor clams live on muddy soil of the coast, flood tide and ebb tide can wash away TBT to other places. Besides, mariculture areas of those shellfish species are the shallow water, which TBT can be degraded by sunlight. Hence, the aquatic organisms might consume some part of TBT which disperses around there and some part of TBT accumulates in sediment.

However, TBT contamination in undulated surf clams, marine water, and sediment from the aquaculture area were found in the highest water level. Considering that sources of TBT might come from high boating activities along Mae Klong River or wastewater discharged from the industries. Due to the fact that after TBT leaches from the source, some of them might attach with particulate matter and settle down to bottom of the sea. Some part might diffuse in the water, and then it will be degraded by UV radiation. Undulated surf clams live under the sea floor might consume TBT while they were feeding. Therefore, TBT contamination in undulated surf clams was different from two other benthic animals. Figure 4.10

demonstrated TBT concentrations in four shellfish species including marine water, and sediment from the different aquaculture areas. It showed that the distance of mariculture site from the shore correlated to TBT concentration in those three mediums. In addition, the contaminated shellfish were concerned that they might contain some TBT which toxic to the consumers since they play an important role as a food source for fish and humans.

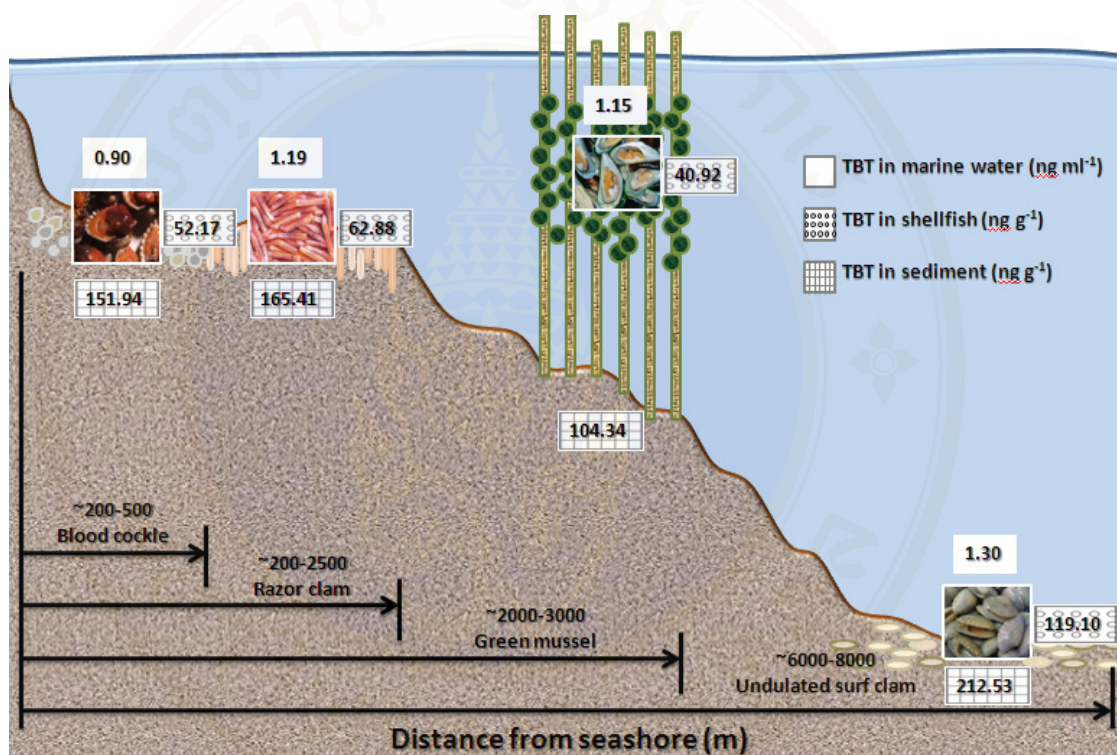


Figure 4.10 TBT contaminations in shellfish, marine water, and sediment in different aquaculture areas

4.3 Evaluation of Human Health Risks

From the result, the highest TBT concentrations in green mussels, razor clams, blood cockles, and undulated surf clams under this study were 73.56 ng g^{-1} dry weight (equivalent to 5.98 ng g^{-1} wet weight), 90.47 ng g^{-1} dry weight (equivalent to 11.26 ng g^{-1} wet weight), 112.16 ng g^{-1} dry weight (equivalent to 17.39 ng g^{-1} wet weight), and 119.10 ng g^{-1} dry weight (equivalent to 10.72 ng g^{-1} wet weight), respectively. With the average of shellfish consumption (expressed in g day^{-1}) and the highest TBT concentration in shellfish (expressed in ng g^{-1} wet weight), daily

exposure was calculated for the consumer with the average weight of 60 kg (Table 4.4). In view of the least daily shellfish consumption rate for the population in Thailand of about 3.55 g day^{-1} , daily intake of TBT in green mussels, undulated surf clams, razor clams, and blood cockles were $0.0004 \text{ } \mu\text{g kg}^{-1}$, $0.0006 \text{ } \mu\text{g kg}^{-1}$, $0.0007 \text{ } \mu\text{g kg}^{-1}$, and $0.0010 \text{ } \mu\text{g kg}^{-1}$ wet weight, respectively. The estimated TBT daily intakes of the TDI ($0.25 \text{ } \mu\text{g kg}^{-1}$) were about 0.14 %, 0.25 %, 0.27 %, and 0.41 %, respectively. The result showed that the daily exposure to TBT was lower than the TDI about 250-625 times. It could be assumed that if the consumer eats 3.55 g of shellfish per day, it is safety for human health.

In addition, if the daily consumption of 71 g day^{-1} , daily intake of TBT in green mussels, undulated surf clams, razor clams, and blood cockles were $0.0071 \text{ } \mu\text{g kg}^{-1}$, $0.0127 \text{ } \mu\text{g kg}^{-1}$, $0.0133 \text{ } \mu\text{g kg}^{-1}$, and $0.0206 \text{ } \mu\text{g kg}^{-1}$ wet weight, respectively. The estimated TBT daily intakes of the TDI were about 2.83 %, 5.07 %, 5.33 %, and 8.23 %, respectively. The daily exposure to TBT was lower than the TDI about 12-35 times. It seems likely that although the consumer eats shellfish in the highest consumption rate, it is still safety for human health. However, the above result was just calculated from the average daily consumption of shellfish which is safe for human consumption.

The maximum shellfish consumption which is not exceeded the TDI level and safety for human health was calculated. Daily up take of shellfish consumption in those shellfish species should not more than 0.86 kg for blood cockles, 1.33 kg for razor clams, 1.40 kg for undulated surf clams, and 2.51 kg for green mussels. In addition, TBT concentrations in shellfish based on this study were divided into three categories; high, moderate, and low. The daily consumption of shellfish was inversely proportional to the level of TBT in those shellfish species. Therefore, high TBT levels ranged from $11.26\text{-}17.39 \text{ ng g}^{-1}$ wet weight, daily shellfish consumption ranged from 0.86-1.33 kg. Moderate TBT levels ranged from $6.44\text{-}10.72 \text{ ng g}^{-1}$ wet weight, daily shellfish consumption ranged from 1.40-2.33 kg. Low TBT levels ranged from $2.37\text{-}5.98 \text{ ng g}^{-1}$ wet weight, daily shellfish consumption ranged from 2.51-6.33 kg.

Table 4.4 A variety of daily shellfish consumption and the daily exposure of TBT in shellfish

Percentage	Shellfish consumption (g day ⁻¹)	Green mussel		Undulated surf clam		Razor clam		Blood cockle	
		Daily exposure (µg kg ⁻¹)	Daily exposure percentage of the TDI	Daily exposure (µg kg ⁻¹)	Daily exposure percentage of the TDI	Daily exposure (µg kg ⁻¹)	Daily exposure percentage of the TDI	Daily exposure (µg kg ⁻¹)	Daily exposure percentage of the TDI
5%	3.55	0.0004	0.14	0.0006	0.25	0.0007	0.27	0.0010	0.41
10%	7.10	0.0007	0.28	0.0013	0.51	0.0013	0.53	0.0021	0.82
15%	10.65	0.0011	0.42	0.0019	0.76	0.0020	0.80	0.0031	1.23
20%	14.20	0.0014	0.57	0.0025	1.01	0.0027	1.07	0.0041	1.65
25%	17.75	0.0018	0.71	0.0032	1.27	0.0033	1.33	0.0051	2.06
30%	21.30	0.0021	0.85	0.0038	1.52	0.0040	1.60	0.0062	2.47
35%	24.85	0.0025	0.99	0.0044	1.78	0.0047	1.87	0.0072	2.88
40%	28.40	0.0028	1.13	0.0051	2.03	0.0053	2.13	0.0082	3.29
45%	31.95	0.0032	1.27	0.0057	2.28	0.0060	2.40	0.0093	3.70
50%	35.50	0.0035	1.42	0.0063	2.54	0.0067	2.66	0.0103	4.12
55%	39.05	0.0039	1.56	0.0070	2.79	0.0073	2.93	0.0113	4.53
60%	42.60	0.0042	1.70	0.0076	3.04	0.0080	3.20	0.0123	4.94
65%	46.15	0.0046	1.84	0.0082	3.30	0.0087	3.46	0.0134	5.35
70%	49.70	0.0050	1.98	0.0089	3.55	0.0093	3.73	0.0144	5.76
75%	53.25	0.0053	2.12	0.0095	3.81	0.0100	4.00	0.0154	6.17
80%	56.80	0.0057	2.26	0.0101	4.06	0.0107	4.26	0.0165	6.59
85%	60.35	0.0060	2.41	0.0108	4.31	0.0113	4.53	0.0175	7.00
90%	63.90	0.0064	2.55	0.0114	4.57	0.0120	4.80	0.0185	7.41
95%	67.45	0.0067	2.69	0.0121	4.82	0.0127	5.06	0.0195	7.82
100%	71.00	0.0071	2.83	0.0127	5.07	0.0133	5.33	0.0206	8.23

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The optimization and validation of analytical methods under this study gave a satisfied result. It confirmed that 20 ml of (9:1) hexane:diethyl ether mixture is the proper eluent which separated nearly 100 % of TBT from other compounds in silica gel column. Liquid-liquid extraction with hexane was done for the purpose of transference of TBT from aqueous phase to organic phase in order to avoid the violence of the reaction between aqueous phase and Grignard reagent. The efficiency of liquid-liquid extraction was about 95 %. Method validation of TBT analysis in shellfish, marine water, and sediment samples also showed the great efficiency. Extraction efficiency and percent recovery of TBT analytical methods for all types of samples were about 87 % and 90 %.

The optimum condition of analytical methods was applied for marine samples. The highest TBT concentration was found in blood cockle (112.16 ng g^{-1} dry weight as Sn) and the lowest level was found in green mussel (26.09 ng g^{-1} dry weight as Sn) collected from Don Hoi Lot Market in November, 2008. Due to TBT concentration in shellfish represents the contamination of TBT in the aquaculture area. It might be assumed that the aquaculture area of blood cockles collected from Don Hoi Lot has more pollution than the aquaculture area of blood cockles collected from Maha Chai because TBT level in cockles collected from Don Hoi Lot Market was higher than TBT level in cockles collected from Maha Chai Market. On the other hand, mariculture site of green mussels collected from Maha Chai has more pollution than mariculture site of green mussels collected from Don Hoi Lot because TBT level in mussels collected from Maha Chai Market was higher than TBT concentration in mussels collected from Don Hoi Lot Market.

The highest TBT concentrations was found in undulated surf clams (119.10 ng g^{-1} dry weight), marine water (1.30 ng ml^{-1}), and sediment (212.53 ng g^{-1})

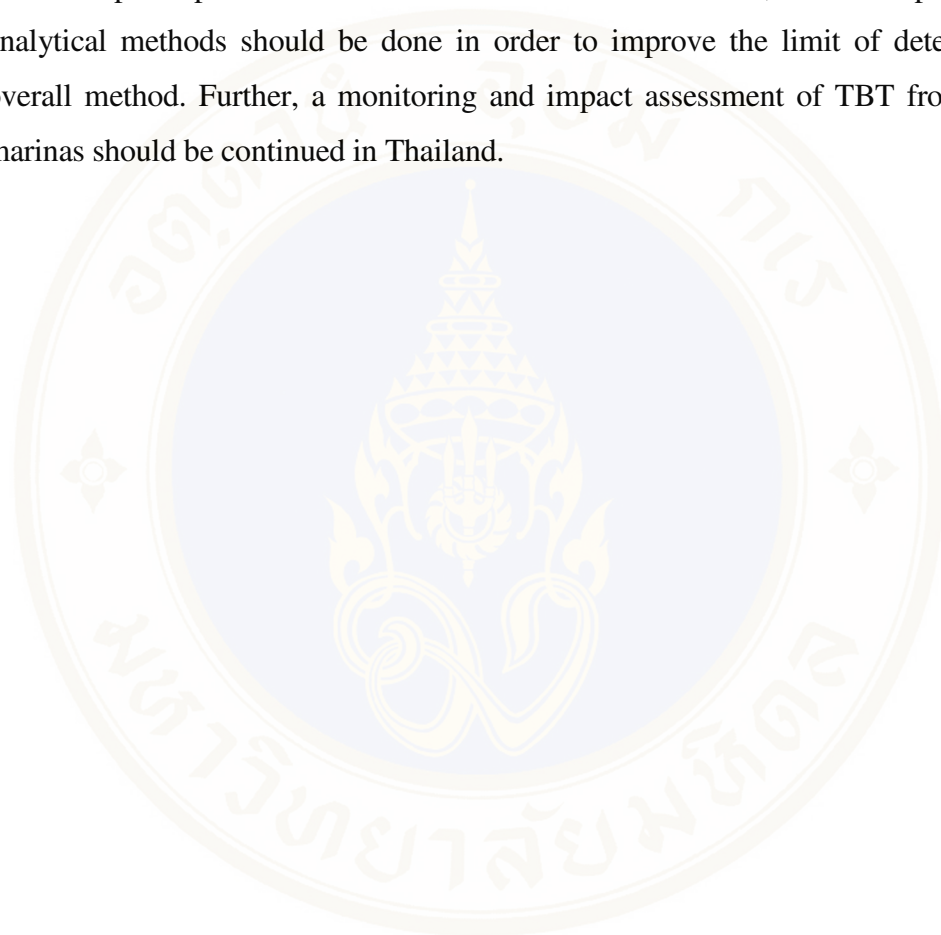
dry weight) collected from Mae Klong River, Samut Songkhram Province in May-June, 2010. Considering the fact that in 2010 Mae Klong River was classified to the less level from the classification in 1994 which means that the water has more pollution than the past. It might be assumed that the illegal discharge of wastewater which contains TBT from the cooling pipe of the industries or the leaching of TBT from the vessels diffuse in marine water. Subsequently, it accumulates in the sediment through the year pass by and effects to an aquatic organisms living in marine environment.

The correlation of TBT contamination in shellfish, marine water, and sediment samples were found that the lowest TBT concentration was detected in marine water and the highest TBT level was detected in sediment. Due to the fact that, when TBT diffused in marine water some of them dissolved or absorbed with microorganisms, detritus or inorganic materials and settled to the sediment layer. Some suspended TBT might be degraded by sunlight easier than the complex of TBT in sediment. As the consumer in marine ecosystem, shellfish can be affected by TBT contamination in marine water and sediment during their life cycle.

The possible risk to human health was assessed on the basis of the consumption of shellfish species sampled in the mariculture areas along the coastal northernmost part of the Gulf of Thailand. Daily exposure was calculated for the consumer with the average weight of 60 kg. The result showed that either the consumer eats shellfish only 3.55 g day⁻¹ or 71 g day⁻¹ per species, it is still safety for human health because the daily intake to TBT was lower than the TDI. In addition, the limit of shellfish consumption which is not exceeded the TDI level and safety for human health was estimated. The maximum shellfish consumptions per day for blood cockles, razor clams, undulated surf clams, and green mussels were 0.86 kg, 1.33 kg, 1.40 kg, and 2.51 kg, respectively. According to the daily consumption of shellfish was inversely proportional to the level of TBT in those shellfish species. The highest TBT level in shellfish based on this study was 17.39 ng g⁻¹ wet weight, maximum daily shellfish consumption should be 0.86 kg. Nevertheless, the lowest TBT level in shellfish was 2.37 ng g⁻¹ wet weight, maximum daily shellfish consumption should be 6.33 kg.

5.2 Recommendation

Although several developed countries have already banned TBT completely, the restrictions on the use of TBT antifouling paints in Thailand have not available yet. The results from the prior studies and this research demonstrated that TBT is a widespread problem in coastal areas of Thailand. Hence, the development of TBT analytical methods should be done in order to improve the limit of detection of the overall method. Further, a monitoring and impact assessment of TBT from ports and marinas should be continued in Thailand.



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Calibration for TBT Concentration

The standard solution of derivative TBT (pentylated TBT) with concentration 100, 200, 300, 400, and 500 ng ml⁻¹ were prepared with the same methods as samples analysis and injected to GC-MS in order to create the calibration curve. The linear calibration curve with regression coefficients 0.991 was observed (Figure A-1). The spectrum of TBT was observed by scan mode and resulted in many different fragments which had a base peak of m/z 179 and 305 (Figure A-2).

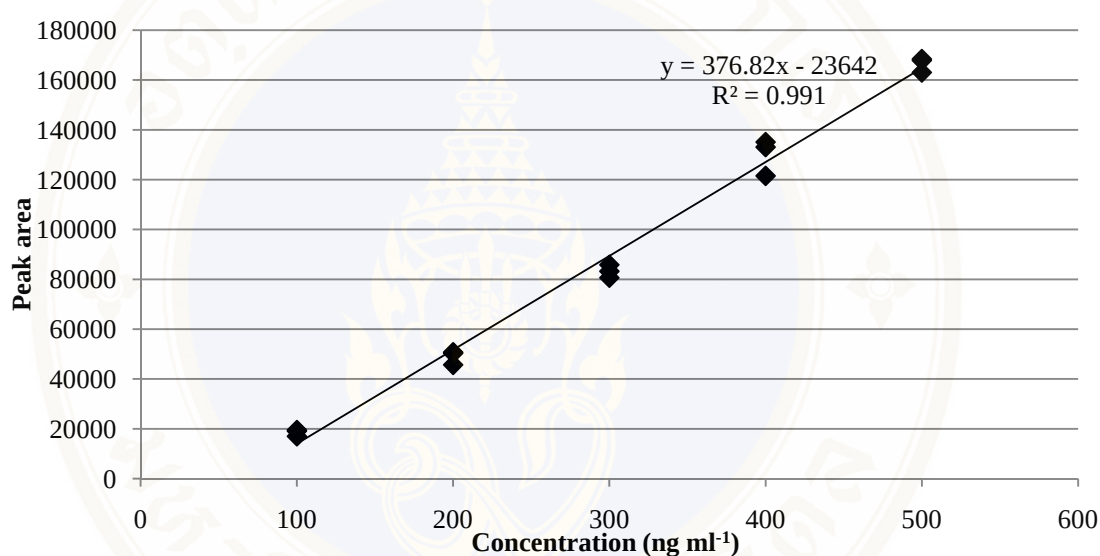


Figure A-1 The calibration curve of TBT standard for the first sampling

The result of TBT in each shellfish sample collected from seafood market (the first sampling) was detected by GC-MS and shown in the chromatogram of abundance and time (Figure A-3). The retention time was 9.093 min. The value was compared with calibration curve to calculate the TBT concentration in sample by using computer program.

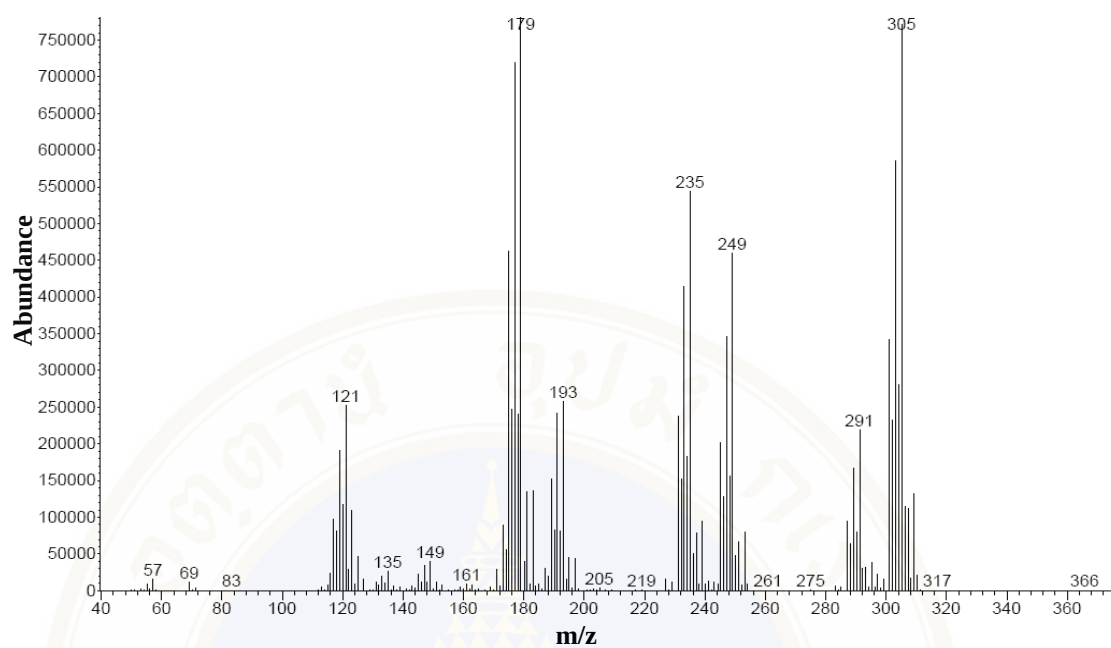


Figure A-2 Mass spectra of pentylated TBT

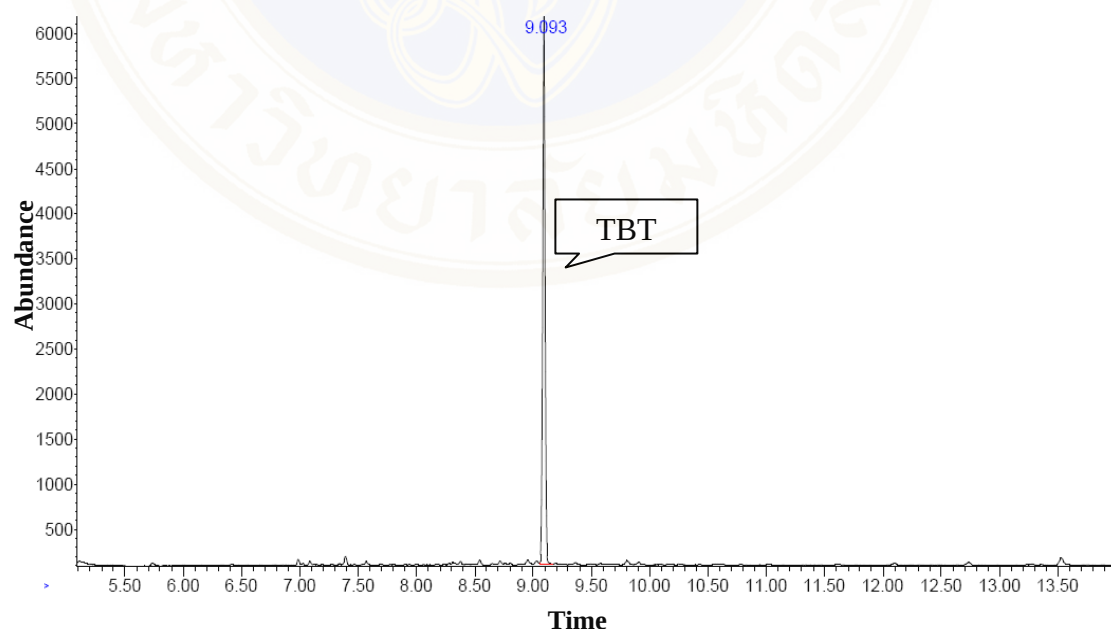


Figure A-3 Chromatogram of pentylated TBT

Figure A-4 showed calibration curve in the concentration ranges as follows: 7.8125; 15.625; 31.25; 62.5; 125; 250; and 500 ng ml⁻¹. The linear calibration curve with regression coefficients 0.998 was observed. TBT concentrations in shellfish, marine water, and sediment samples collected from mariculture areas (the second sampling) were computed and compared with the calibration curve.

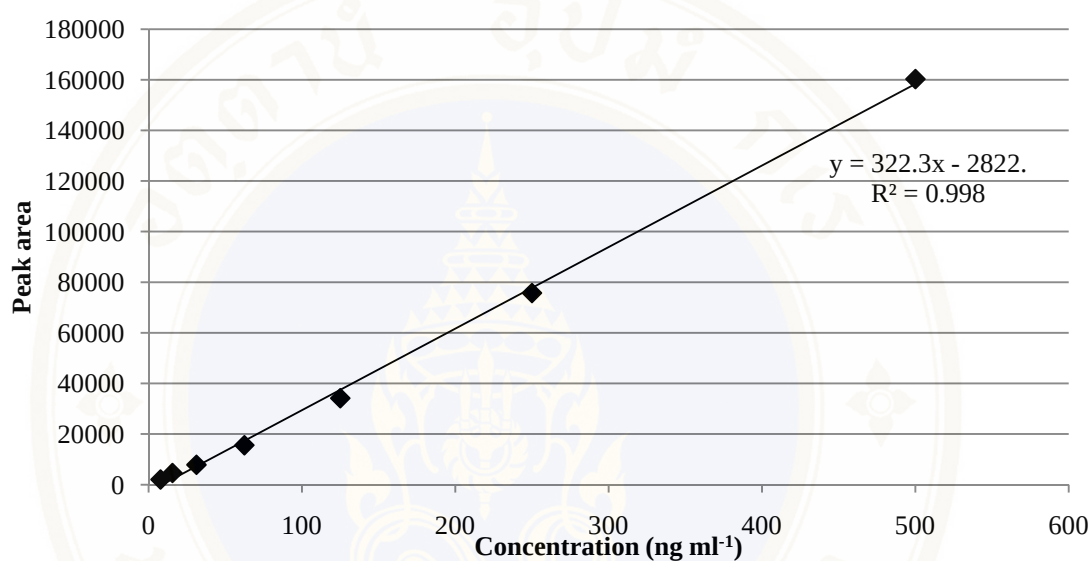
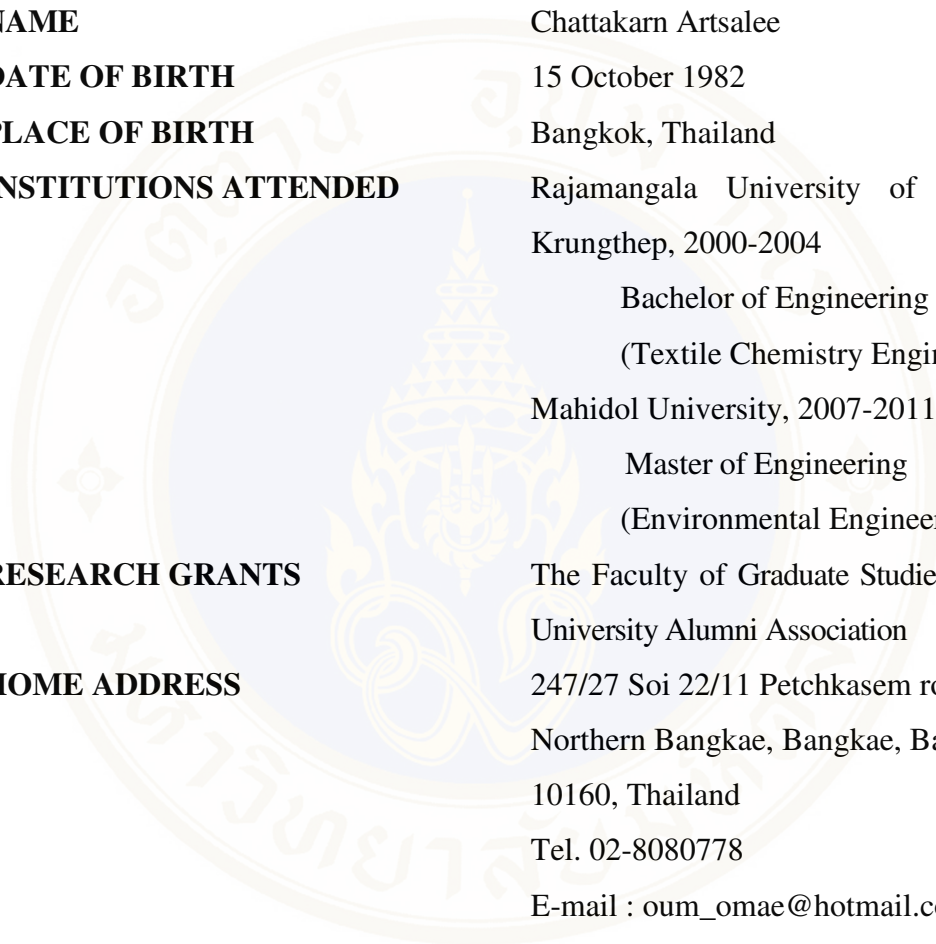


Figure A-4 The calibration curve of TBT standard for the second sampling

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