CHAPTER II

LITERATURE REVIEW

Aquatic environment

Aquatic environment is rather complex and diverse. It includes several distict ecosystem types - (freshwater streams, lakes, ponds, and rivers; estuaries; and marine coastal and deep ocean waters). Each ecosystem type is composed of many different biotic and abiotic components. Biota or living component includes plants, animals, and microorganisms that inhibit specific ecological niches in each ecosystem whereas, the abiotic or nonliving component include physical environment, (e.g., water, substrate, and suspended material) within the boundaries of the ecosystem. Each aquatic ecosystem is therefore a product of complex interactions of living and nonliving components.

The physico-chemical properties of aquatic ecosystems have a profound effects on xenobiotics and their biological activity and an impact of chemical substances to the biotic components. The vulnerable effects of chemicals to the aquatic environment depends on several important factors, including (1) physical and chemical properties of the chemical and its transformation products; (2) concentrations of the chemical entering the ecosystem; (3) duration and type of inputs (acute and chronic, intermittent spill or continuous discharge); (4) properties of the ecosystem that enable to resist changes resulted from the presence of the chemical (e.g., pH buffering capacity of seawater) or to return to its original state after the chemical is removed from the system (e.g., flushing of water from estuaries by tidal action); and (5) location of the ecosystem related to the release site of the chemical.

Based on the fact that the indegenous species of animals, plants, and microorganisms are generally inescapably immersed in the water medium throughout their lives. This is significant since aquatic ecosystems may serve as reservoirs or sinks for various chemicals (Rand and Petrocelli, 1985).

Toxicity testing

Aquatic toxicology has been defined as the studies of effects of chemicals and/or other foreign agents on aquatic organisms with special emphasis on adverse or harmful effects. Toxicity tests are used to evaluate the concentrations of the chemical and the duration of exposure required to produce the criterion effect. Effects of chemical may be in such a minor significance to aquatic organisms which is able to carry on its functions in a normal or additional stress conditions (e.g., changes in pH, dissolved oxygen, and temperature). More effects of chemicl substances to living organisms may also result from the interaction of small amounts of some chemicals and large amounts of other chemicals without any additional stresses.

Theorethically, aquatic toxicity tests are used to detect and evaluate the potential toxicological effects of chemicals on aquatic organisms. Since, these effects are not necessarily harmful, a principal function of the tests is to identify chemicals that can have antagonistic effects on aquatic organisms. Moreover, the tests also provide a data base that can be used to assess the risk strategies in which the chemical agent, the organism, and the exposure conditions are defined.

The aquatic toxicity test is typically called a bioassay. An appropiate definition of bioassay is "a test to evaluate the relative potency of a chemical by comparing its in vitro effect on a living organism with that of a standard preparation". A bioassay is performed to determine the strength of the chemical from the degree of response elicited from the test organisms, not to estimate the *in vivo* concentration of the chemical which is toxic to those organisms. A toxicity test is then performed to measure the degree of response produced by a specific level of chemicl stimulus (Rand and Petrocelli, 1985). A variety of toxicity test methods has been developed by the American Public Health Association (APHA), U.S. Environmental Protection Agency (U.S. EPA), American Society for Testing Materials (ASTM), and Organization for Economic Cooperation and Development (OECD) to evaluate the hazard and potential toxicity of various materials to aquatic organisms.

Tributyltin compounds are currently one of the most well studied chemicals in aquatic toxicity tests, because of their highly toxic levels to organisms in the environment.
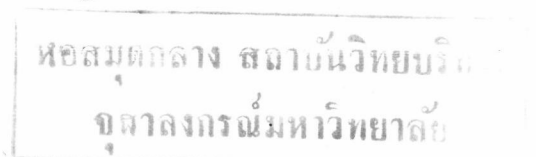
Tributyltin compounds

Tributyltin (TBT) is an active ingredient of several products that act as biocides against a broad range of organisms. It is primarily used as antifouling paint on ship and boat hulls, docks, fishnets, and buoys. Nevertheless, it has been reported to discourage the growth of marine organisms such as barnacles, bacteria, tubeworms, mussels and algae.

TBT *per se* is unstable substance. It will break down in the environment unless it is combined with the other element such as oxygen. Within the group, bis (tributyltin) oxide , or TBTO is one of the most common used. As a result, TBTO has been widely used as a representative of TBT compounds in toxicity test. (Pesticide Information Project, 1993).

Identity of tributyltin compounds

Tributyltin compounds are organic derivatives of tin (Sn^{IV}) characterized by the presence of covalent bonds between three carbon and a tin atoms. The conformation following general formula is $(n-C_4H_9)_3Sn-X$, where X is an anion or a group linked covalently through a hetero-atom.

It should be noted that the nature of X influences the physico-chemical properties, particularly the relative solubility in water and non-polar solvents, and the vapour pressure.

These compounds differ from inorganic tin in both effects and function. An important member of the group is tributytin oxide (TBTO). Other industrially important tributyltin derivatives include tributyltin fluoride, tributyltin methacrylate (monomer or copolymer), tributyltin naphthenate, and tributyltin phosphate. The identity of tributyltin compounds is shown in Table 2.1.

Table 2.1 Identity of tributyltin compounds

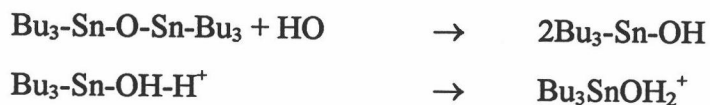| Common name:	Tributyltin oxide
Chemical names:	
IUPAC:	Distannoxane; hexabutyl;
CAS:	bis (tributyltin) oxide
Synonyms and trade names:	Bis (tributyltinoxide) of tin
	bis (tributyl-stannyl) oxide;
	BTO; Butinox; ENT 24979;
	Hexabutyldistannoxane;
	Oxybis (tributyltin) oxide; Tin
	Oxybis (tributyl)
Molecular formular:	$C_{24}H_{54}OSn_2$
Registry numbers:	CAS: 56-35-9
	RTECS: JM 8750000

Source : International Register of Potentially Toxic Chemicals (IRPTC), 1992

Physico-chemical properties of tributyltin compounds

TBTO is a mild oxidizing agent. It is flammable but does not form explosive mixture with air. It reacts quantitatively, however, with bromide or iodine at room temperature by cleavage of the Sn-O bond.

In the presence of oxygen, light and heat, slow breakdown occurs with the formation of tetra-n-butyltin, di-n-butyltin oxide, and finally tin(IV)oxide by dealkylation. This degradation may be inhibited by an addition of 0.1-1.0 % of stabilizers.

Laughlin (1986) showed that TBTO can react with normal constituents of seawater in the following ways:

$$Bu_3\text{-}Sn\text{-}O\text{-}Sn\text{-}Bu_3 + HO \quad \rightarrow \quad 2Bu_3\text{-}Sn\text{-}OH$$

$$Bu_3\text{-}Sn\text{-}OH\text{-}H^+ \quad \rightarrow \quad Bu_3SnOH_2^+$$

$$Bu_3\text{-}Sn\text{-}OH + CO_3^{2-} \quad \rightarrow \quad Bu_3SnCO_3^- + OH^-$$

$$Bu_3\text{-}Sn\text{-}OH_2 + Cl^- \quad \rightarrow \quad Bu_3\text{-}Sn\text{-}Cl + H_2O$$

The predominant forms are $Bu_3SnOH_2^+$ and Bu_3SnCl at pH < 7, Bu_3SnCl, Bu_3SnOH and $Bu_3SnCO_3^-$ at pH 8, and Bu_3SnOH and $Bu_3SnCO_3^-$ at pH > 10.

Under normal conditions in sea water, it is considered that the three forms of TBTO (hydroxide, chloride, and carbonate) are in an equilibrium. Some physical and chemical properties of tributyltin oxide is summarized in Table 2.2.

Table 2.2 Some physical and chemical properties of tributyltin oxide

Description:	Colourless liquid with characteristic odor
Melting points:	- 73° C
Boiling points:	87° C
Relative density at 20 ° C:	1.17 to 1.18
Refractive index at 20 ° C	1.4880 - 1.4895
Solubility:	Low, varying between < 1.0 and > 100 mg/litre at different temperatures and pH. Soluble in lipids and highly soluble in some organic solvents (ethanol, ether, halogenated hydrocarbon, etc.)
Adsorbtion coefficients:	Between 110 - 55000
The octonal/water partition coefficient (log P_{ow})	Between 3.19 - 3.84 for distilled water and 3.54 for seawater

Source : IRPTC, 1992; World Health Organization (WHO), 1990

Toxicology and mode of action of triorganotin compounds

Toxicological patterns of organotin compounds are extremely complex. However, progressive introduction of organic groups at the tin atom in any R_nSnX_{4-n} series generally produce a maximum biological actively when n=3 (i.e., for the triorganotin compounds, R_3SnX derivatives generally have very little effect on changes

of the biological activity). Within any R_3SnX series, the activity or toxicity of triorganotin compound , is resulted from the three organic groups (Table 2.3). For example, in the tri-n-alkyltin series, the trimethyltins have the highest toxicity to insects and mammals, the tripropyltins to gram-negative bacteria, and the tributytins to gram-positive bacteria and fungi. Further increase in the n-alkyl chain length produces a dramatic decrease in biological activity. Accordingly, the trioctyltin compounds are essentially non-toxic to all living organisms.

The tetraorganotins, R_4Sn, is found to exhibit a delayed toxic action, which may be due to their *de novo* conversion to triorganotin compound (R_3SnX) in the organisms.

Table 2.3 Species specificity of triorganotin compounds, R_3SnX

Species	R in most active R_3SnX compound
Insects	CH_3
Mammals	C_2H_5
Gram negative bacteria	$n-C_3H_7$
Gram positive bactria, fish,fungi, molluscs, plants	$n-C_4H_9$
Fish, fungi, molluscs	C_6H_5
Fish, mites	cyclo-C_6H_{11}
	$C_6H_5(CH_3)_2CCH_2$

Source : Blunden and Chapman (1986)

The biological activity of organotin compounds is believed to be resulted from their ability to bind to certain proteins, although the exact nature of the binding site is not known. The triorganotin derivatives specifically interfere mitrochondrial functions, which can be observed as follows :

    (a) by interaction with mitochondrial membranes causing swelling and disruption of mitochondria,

(b) by secondary effects derived from their ability to act as ionophores to derange mitochondrial function through mediation of chloride-hydroxide ion exchange across the lipid membrane, and

(c) by their ability to inhibit the fundamental energy conservation processes involved in the synthesis of adenosine triphospate, in which living organisms share many common features. This oxidative phosphorylation process is able to be inhibited by triorganotins (Blunden and Chapman, 1986).

Sources of environmental exposure of tributyltin compounds

For TBT antifouling paint use, estuaries receive TBT from two primary sources ; sites of application and sites of use. TBT paints are generally applied to the underwater hull areas of boats and ships in private boat yards or commercial shipyards. The sites of use are any waters where boats and ships travelled. Accordingly, TBT paint use patterns can vary significantly.

Recreational boaters and owners of small commercial vessels probably paint their own hulls. They may carry out at small boat yards, whereas large commercial vessels need the efficiency services of graving or floating drydocks in commercial shipyards. Therefore, TBT sources from sites of application range from small boat yards to large shipyards.

Sites of TBT paint use are similarly varied. Recreational boats usually spend 95 percent, or more of the time at piers or mooring while spending 5 percent or less of the time underway or anchored in open water. Local commercial vessels may spend 50 percent of the time at pierside and the remaining time in open water to conduct their business. However, large-ocean-going vessels spend most of their time at sea. During idle periods, their vessels are moored in designated anchorages, or continuous but variable magnitudes near piers and marinas (Bailey, 1986).

Global production of organotin compounds was on an order of 40,000 metric tons per year accounted about 7-8 percent of the tin used each year. Of all

commercially available metals, tin has the greatest number of organometallic compounds in use. The amount of the triorganotin compounds used as biocides (approximately 8,000 tonnes world-wide annually) were exceeded by the non-toxic applications of the di- and monoorganotin derivatives (Blunden and Chapman, 1986).

Modes of entry of triorganotin compounds to the environment

There are a variety of pathways which can be envisaged for the entry of triorganotin compounds into the environment. Table 2.4 shows the possibilty modes of TBT into the environment.

Table 2.4 Possible direct modes of entry of TBT compounds into the environment.

Medium	Source
Air	- Agricultural spraying
	- Volatization from biocidal treatments
	- Antifouling paint sprays
	- Incineration of organotin treated or stabilized waste materials
Soil	- Agricultural applications
	- Wood preservation
Water	- Antifouling coatings
	- Molluscicides
	- Overspray from agricultural applications
	- Land run-off from agricultural usage
	- Industrial processes, e.g. slimicides in paper manufacture

Source : Blunden and Chapman (1986)

<u>Applications of the tributyltin compounds (R_3SnX)</u>

Tributyltin compounds can be applied for;

- Agriculture (fungicides, antifeedants and acaricides)
- Antifouling paint biocides
- Wood preservative fungicides
- Stone preservation
- Disinfectants
- Molluscicides
- Homogeneous catalysts for RTV silicones, polyurethane foams and tranesterification reactions. (Blunden and Chapman, 1986)

Environmental transport and transformation of tributyltin compounds

Once released into the environment , TBT appears to be removed from the water column principally by adsorbing to lipids and particulate matters and alternatively through assimilation and metabolism by plants and animals. It appears readily susceptible to degradation via photolysis and a *de novo* mechanism of various organisms (Cardwell and Sheldon, 1986).

<u>Adsorption onto and desorption from particles</u>

When TBTO (and TBT) is introduced into water, its partitioning will occure. This is caused by its lipophilic character and poor water solubility resulting in leaving the aqueous phase and preferentially adsorb onto particles (WHO, 1990 from Hinga *et al.*, 1987)

Langston and Pope (1995) studied the influence of some major physico-chemical factors which controlled TBT partitioning, using ^{14}C-TBT batch testing methods and natural sediment/water systems. The result showed that the partitioning exhibited a TBT concentration dependence. Moreover, partitioning was also influenced by salinities (lowest K_d values occuring at low to intermediate salinities) and

pH (highest K_d values were observed at circum-neutral pH). More importantly, TBT appeared to show characteristics of both metal ions hydrophillic and hydrophobic materials in correlation with the parameters described above. Sediment characteristics also influenced partitioning; K_d was positively correlated with total organic material, humic substances, Mn^{++}, and a high silt content. Stang and Seligman (1987) found that there was apparently no desorption of TBTO itself but dibutyltin derivatives formed by desorbed degradation with the varying rates between 0.16 and 0.55 ng DBT/cm^2 per day from Pearl Harbour sediment.

The adsorption may depend on many diferrent factors, amongst which are as follows :

- salinity;

- nature and size of particles in suspension;

- amount of suspended particles;

- temperature; and

- presence of dissolved organic matter. (WHO,1990)

Degradation

Degradation involves breaking of a Sn-C bond and this can be occurred by a number of different processes including :

1. Ultraviolet (UV) irradiation

2. Biological cleavage

3. Chemical cleavage

4. Gamma (γ) irradiation

5. Thermal cleavage

Of these , γ-irradiation has a little effect on degradation of TBTO in the environment, which is due to its negligible intensity at the earth's surface (Blunden and Chapman, 1986).

A number of studies have shown that a degradation pathway of tributyltin compounds in the environment, involves progressive debutylation. Theoretically, it is completed with the sequential release of tin oxide (SnO_2) into water.

$$R_3SnX \rightarrow R_2SnX_2 \rightarrow RSnX_3 \rightarrow SnX_4$$
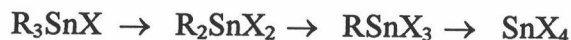
Seligman *et al*. (1988) reported that the half-life of TBT in water from the Shelter Island Yacht Marina was six days. The half-life of ^{14}C-TBT (2 μg/L) added to yacht basin water was 6 and 7 days in the light and dark condition, respectively. Estimated half-life of biological degradation of tributyltin in freshwater and sediment in Canada varied from a few weeks to 4-5 months (Maguire, 1992). Biodegradation and the photolysis paths of butyltin compounds in natural waters were recently examined by Watanabe *et al*. (1992). It was found that TBT biodegradation in an unfiltered seawater was fast and the half-life was about a week, but pretreatment with glass fiber made the half-life of TBT longer to be about 80 days. Rapid photolysis of TBT in seawater by sunlight was also observed.

Dowson *et al*. (1993a) investigated the degradation of tributyltin (TBT) in contaminated freshwater and estuarine sediments for 330 days under controlled laboratory conditions. Only a little difference in degradation rates between freshwater and estuarine sediments was reported. Rates of TBT degradation at different depths were illustrated using regression modelling. The revealed TBT half life ranges from 360-775 days in surficial sediments.

De Mora *et al*. (1995) found that marina with TBT-contaminated sediments near Auckland, New Zealand deposited over 15 years ago. The interpretation from their simple model indicated that degradation occured with the first-order kinetics and TBT in marina sediments had a half-life of about 2.5 years.

Bioaccumulation and elimination

The lipophilic properties of TBTO and its moderately high octanol-water partition coefficient (log P_{ow} > 3) contribute bioaccumulation in living organisms. Davies *et al*. (1986) studied the accumulation of total tin and tributyltin from antifouling paint using oysters (*Crassostrea gigas*) and scallops (*Pecten maximus*) and the subsequent depuration of these substances in the suspended nets for 31 weeks in Loch Moidart, W. Scotland. From the results monitored over a total period of 41

weeks, it was found that oysters accumulated up to 1.41 mg tin/kg (0.87 mgTBT/kg) and subsequently 90% of this was lost during depuration. Juvenile scallops accumulated 2.5 mg/kg total tin (1.86 mgTBT /kg), only 20-40% of this was lost.

Rice and Short (1989) studied accumulation and elimination of tributyltin (TBT) in blue crabs (*Callinectes sapidus*) after 16-day fed with TBT-contaminated prey. Crabs were fed with grass shrimp (*Palaemonetes pugio*) contaminated with 1.8 µgTBT, 0.09 µgDBT and 0.03 µgMBT per gram of wet weight tissue. The experimental crabs consumed about 2.02 µg of TBT daily. TBT was sequentially debutylated in a significant level by *C. sapidus*. The highest TBT concentration detected in the blue crab was at 0.12 µg/g after 4 days of feeding. The highest DBT and MBT concentrations were found at 0.39 µg/L (at 8 days) and 0.35 µg/L (at 12 days), respectively.

Kim *et al*. (1996) measured butyltin (BT) concentrations of Steller sea lions collected from coastal waters of Hokkaido, Japan. With the exception of hair, BT concentrations were an order of magnitude higher than that in tissues and organ including liver. There was no relationship observed between BT concentrations and the lipid content in any tissues. Results also suggested that 26% of the total BT burden in the body was eliminated through shedding. Selective accumulation of BTs in liver and hair was attributed to its protein-binding capacity rather than lipophilicity. Discovery of high concentrations of BTs in hair implied their excretion by shedding in piliferous animals.

Environmental concentrations of tributyltin compounds

Levels of tributyltin (TBT) in water, sediment, and biota are elevated within the proximity of marinas, commercial harbours, cooling systems and fish nets and cages treated with TBT-based antifoulant paints. Hazadous levels of TBT were found in estuaries and coastal waters in various parts of the world, but such a research has not been reported in Thailand.

Water and sediment

The hazard posed by tributyltin to an organism in water or sediment may regard a function of its toxicity, concentrations and persistence in water or sediment (Maguire, 1992).

Cleary and Stebbing (1985) monitored organotin concentrations in water from various sites along the southwest coast of England during 1984. It was found that levels of organotin compounds were below detection limits (0.1 µgTBTO/L) at all marina sites, but higher concentrations were found in harbour waters, where the highest value being 0.88 µgTBTO/L at Plymouth (Sutton harbour). In the other study, it was reported that TBT concentrations in waters of Poole Harbour ranged between 2-139 ng/L in marinas. Benthic sediments decreased from 0.52 to 0.02 µg/g at the harbour mouth were found (Langston et al.,1987).

Hall et al. (1987) measured TBT concentrations from microlayer in the Maryland portion of the Chesapeake Bay. The concentrations ranged from 54-1171 ng/L in marinas, whereas in the microlayer of a heavily used shipping channel were of 41 and 29 mg/L.

Alizieu et al. (1989) measured seawater samples in 1986-1987 at different locations of the French Atlantic coast, including marinas and oyster culture areas. TBT concentrations were in the range of < 2-1500 ng/L. TBT compounds in surface seawater from marinas in Greece were between 25 to 423 ng/L (as Sn) (Fytianos and Samanidou,1990).

Ritsema and Laane (1991) collected surface water samples taken from various locations in 26 marinas/harbour in the Netherlands in June and August 1989 for measuring organotin concentrations. The concentrations of TBT, DBT, and MBT found were 0.1-7210, 0.1-1340 ,and 0.1-460 ng/L, respectively.

Dowson et al. (1992) measured sediments and water column of seven Suffork and Essex estuaries in the UK. TBT concentrations in water column and sediment ranged from < 3-71.2 ng/L, and < 3-3935 ng/g, respectively. The highest tributyltin concentrations was found at sites with high levels of boating activity.

Hasan and Juma (1992) determined TBT in seawater and sediment from in and around marinas from of coastal stations in Bahrain. The TBT in seawater ranged from 2.29-17.88 μg/L, while a TBT concentration of 128-1930 ng/g was obtained in sediment around marinas. At the same locales, TBT concentration found in seawater and sediments were 3.42-8.35 μg/L and 352-1330 ng/g, respectively. Moreover, the water samples collected from the Gulf of Naples, Italy, contained TBT at the concentration between 0.01-0.56 μg/L (Cocchieri et al.,1993).

Cortez (1993) found that TBT concentrations in sediments from four different Portuguese coastal environments ranged from 1-17 ng/g in the areas with good water exchange with the sea, 328 ng/g in a lagoon with poor water exchange, 220 ng/g near a shipyard, and 520 ng/g from the discharged water from shipyards. Surprisingly, TBT in sediments from the southern Chesapeake Bay in a marina were as high as 4000 μgTBT/kg sediments (Espourteille et al.,1993).

After French restrictions on TBT in 1982, 14 sediment cores samples from the Arcachon Bay were determined in 1990 . It was found that the use of tin-containing boat paints was still in considerable amount. The concentrations of TBT in the sediment varied from below the detection limit (0.6-1.2 ng/g) in mariculture areas to more than 600 ng/g in Andernos and Larros Harbour (Sarradin et al.,1994).

Ko et al. (1995) reported that TBT concentrations up to 53 μg/g and 18.3 μg/g were found in sediment at positions underneath vessels hoists in the Causeway Bay and Aberdeen Marinas, Hong Kong. The average concentration of TBT from all sites in local marinas and shipyards was about 500 ng/g. TBT concentration of the sediment samples from intertidal locations in Portland and Boothbay, Maine (USA) in 1990 and 1992 ranged from 24 to 12,400 ng/g (dry weight) (Page et al., 1996).

Tolosa et al. (1996) detected subsurface waters from Mediterranean (Côte d' Azur) coastline. TBT was found in all marinas with concentration reaching 460 ng/L.

Tong et al. (1996) reported that the levels of TBT in the coastal environment of the Malaysian Peninsula ranged from < 3.4 to 20 ng/L in seawater in unexposed areas in comparison to that of higher than 30 ng/L in the coastal areas with high boat and ship activities. The highest TBT level found in such areas was 281.8 ng/L TBT in

sediments were found ranging from < 0.7 ng/g dry weight in unexposed coastal sites to as high as 216.5 ng/g dry weight for a site within a port area.

Biota

TBT concentrations found in American oysters, *Crassostrea virginica* collected from the Southern Chesapeake Bay, ranged from 210 µg/kg dry weight while a significantly higher concentration were found in such an animal collected from the Elizabeth River in the Atlantic Coast of Virginia. Futhermore, oyster samples were analysed for TBT contamination, from 73 different sites along the Gulf of Mexico collected in winters of 1989, 1990, and 1991. The mean concentrations were 85, 30, and 43 ng Sn/g for 1989, 1990 ,and 1991, respectively (Garcia-Romero *et al*, 1993). It was also found that TBT level was as high as 0.7 µg/g in adult scallops (*Pecten maximus*) in the North Water of Mulroy on the north coast of Irland (Minchin *et al.*, 1987).

Wild blue mussel (*Mytilus edulis*) under natural conditions (Rungsted marina, Denmark) were studied for the accumulation of organic tin in a total period of 51 days. The concentration of total tin in mussel tissue was 5.14-29.39 µg dry weight and at 0.06-0.08 µg Sn/L in water. The bioconcentration factors were from 5000-60000 (Zuolian and Jensen, 1989). The TBT concentration as high as 314 ngTBT/ Sn g in mussel tissue was recorded by Stewart and Thompson (1994) at the mouth of the Fraser River in the southwestern coast of the British Columbia.

Bryan *et al.* (1989) measured TBT concentrations of mud snails (*Ilynassa obsoleta*) collected along gradients of tributyltin (TBT) pollution in the York River-Sarah Creek region of the Chesapeake Bay. TBT concentration in the tissues ranged from 20 ng/g to 620-730 ng/g dry weight. TBT-tissue concentration of molluscs (*Littorina littorea* and *Mya arenaria*) around the north-eastern part of the island of Fyn, Denmark was found between 0.25-14.70 µg/g. The concentration factors for sediment compared to *L. littorea* were 500-9000 (Kure and Depledge, 1994).

Tong *et al.* (1996) reported the levels of TBT in tissue of cockle and softshell clam sampled from local markets in the coastal environment of the Malaysian Peninsular. They were in the range from < 0.5 to 3.7 ng/g wet weight. The levels of

TBT also found in green mussel samples both from the market (23.5 ng/g wet weight) and those from farm (14.2 ng/g wet weight).

Short and Thrower (1986) found TBT residues between 0.28 and 0.90 µg/g in salmon (*Oncorhynchus tshawytscha*) maintained in TBT-treated pens for 3 to 19 months. Subsequently, salmon in American fish markets were further investigated for TBT contamination. The residual TBT was found in the salmon tissue for up to 0.2 µg/g. It was mentioned that cooking does not effectively destroy or remove TBT from salmon tissues.

Davies *et al.* (1987) determined the accumulation of TBT in the Atlantic salmon (*Salmo salar*) reared in netting cages containing tributyltin compounds from Scottish marine salmon cultivation units. After exposure to low concentration (0.1-1.0 µg/L) of TBT in solution, such a higher concentration of this substance was found in various organs. Of which approximately 0.5-1.0 mg/kg TBT was found in muscle tissue.

Iwata *et al.* (1994) collected the blubber samples from eight species (12 specimens) of marine mammals caught between 1981 and 1993 from seas surrounding Japan and in the Indian, North Pacific and Atlantic Oceans for analysing butyltin compounds (BTCs). BTCs were detected in all animals except a minke whale from the Antarctic Ocean. The highest residual levels were found in a finless porpoise from the Seto-inland Sea, Japan with a BTC concentration of 770 ng/g wet weight.

Kannan *et al.* (1995) determined butyltin (BT) residues in mussle tissue of fish collected from local markets and seafood shops in India, Bangladesh, Thailand, Indonesia, Vietnam, Taiwan, Australia, Papua New Guinea, and the Solomon Islands. Butyltin was detected in most samples of fish muscle and liver. The concentrations were about 0.2 to 190 ng/g ,and 1.2 to 570 ng/g wet weight in muscle tissue,and liver, respectively. Butyltin concentrations in muscle tissue of fish from Thailand were then investigated. The fish were collected from two markets in Bangkok in January 1994. The BT concentrations of 2.9, 4.4, and 16 ng/g wet weight, were found in pomfret (*Pampus argenteus*), short-bodied mackerel (*Rastrelliger kanagurta*) and giant seaperch (*L. calcalifer*), respectively.

Kannan *et al.* (1996) analysed butyltin compounds (BTCs) in the carcasses of bottlenose dolphin (*Tursiops truncatus*), bluefin tuna (*Thunnus thynnus thynnus*), and blue shark (*Prionace glauca*) from the Italian coast in the Mediterranean Sea between 1992-1993. The concentration of BTCs in the liver of dolphin (1,200-2,200 ng/g wet weight) were significantly higher than that in the blubber (48-320 ng/g wet weight).

Kim *et al.* (1996) found that TBT concentrations in various tissues and organs of stellar sea lions collected from coastal waters of Hokkaido, Japan was about 1.7 ng/g wet weight (skin) to 1300 ng/g wet weight (hair at breast).

Effects of tributyltin compounds on aquatic organisms

The biological response of aquatic organisms to tributyltin varies enormously. TBT provokes a wide range of harmful effects (sublethal to mortal) to numerous organisms (bacteria to fish) at greatly differing scales (RNA damage to local extinctions). Sensitivity of aquatic species to tributyltin also varies. It has been shown to affect settlement, growth, and mortality of larval; shell deposition of growing; gonadal development and gender of adult oysters and other bivalves. It also causes imposex (the development of male characteristics) in female gastropods. TBT reduces reproductive performance, neonate survival, and juvenile growth rate in crustaceans. The toxicity of TBT to fish is highly variable.

The acute and chronic toxicities of TBT on various aquatic organisms were summerized in Table 2.5, and Table 2.6, respectively.

Table 2.5 Acute toxicity values of tributyltin compounds to various aquatic animals

Organisms	Tributyltin compounds	Effect (Confidence limit)	Types of exposure	Reference
Coelenterate				
Hydra sp. (adults)	TBTO	96-h LC50 = 0.5 μg/L (0.4-0.7)	flow through	Brooke *et al.*, 1986
Annelid				
Lumbriculus variegatus (adults)	TBTO	96-h LC50 = 5.4 μg/L (4.8-6.1)	flow through	Brooke *et al.*, 1986
Gastropod				
Biomphalaria sp.	TBTO	48-h LC50 = 10-100 μg/L	static	Rexrode, 1987
Bivalves				
Mytilus edulis (larvae)	TBTO	48-h LC50 = 2.3 μg/L	static renewal	Rexrode, 1987
Crassostrea giga (larvae)	TBTO	48-h LC50 = 1.6 μg/L	static renewal	Rexrode, 1987
Mercenaria mercenaria	TBTO	48-h LC50 = 1-2.5 μg/L	flow through	Laughlin Jr. *et al.*, 1989
Crustaceans				
Daphnia magna (adults)	TBTO	48-h LC50 = 4.3 μg/L (3.6-5.2)	flow through	Brooke *et al.* 1986

Table 2.5 Acute toxicity values of tributyltin compounds to various aquatic animals (Continued)

Organisms	Tributyltin compounds	Effect (Confidence limit)	Types of exposure	Reference
Daphnia magna (adults)	TBTCl	96-h LC50 = 5.1 µg/L	static	Meador ,1986
Eurytemora affinis (subadults)	TBTCl	72-h LC_{50} = 0.6 µg/L (0.1-0.2)	flow through	Bushong *et al.,* 1988
Acartia tonsa (adults)	TBTCl	48-h LC50 = 1.11 µg/L (0.7-2.2)	flow through	Bushong *et al.,* 1988
Penaeus monodon, (larvae)	TBTO	24-h LC50 = 0.67-3.47 µg/L	static	Prapagdee, 1995
Acanthomysis sculpta (juveniles)	TBT	96-h LC50 = 0.42 µg/L	static renewal	Davidson, 1986
Gammarus pseudolimnaeus (adults)	TBTO	96-h LC50 = 3.7 µg/L (2.9-4.9)	flow through	Brooke *et al.,* 1986
Gammarus sp.	TBTCl	96-h LC50 = 5.3 µg/L (3.3-7.8)	flow through	Bushong *et al.* 1988
Insect				
Culex sp. (larvae)	TBTO	96-h LC50 = 10.2 µg/L (3.5-28.8)	flow through	Brooke *et al.* 1986
Fish				
Salmo gaidneri (juvenile)	TBTO	96-h LC50 = 3.9 µg/L (3.6-4.3)	flow through	Brooke *et al.,* 1986

Table 2.5 Acute toxicity values of tributyltin compounds to various aquatic animals (Continued)

Organisms	Tributyltin compounds	Effect (Confidence limit)	Types of exposure	Reference
Salmo gaidneri (juveniles)	TBTO	96-h LC50 = 1.41 µg/L	flow through	Martin, 1989
Salmo gaidneri (subadults)	TBTO	28-h EC50 = 30.8 µg/L	flow through	Chliamovitch and Kuhn, 1977
Ictalurus punctatus, (juveniles)	TBTO	96-h LC50 = 5.5 µg/L (4.7–6.3)	flow through	Brooke *et al.,* 1986
Ictalurus punctatus (juveniles)	TBTO	96-h LC50 = 12.0µg/L (7.3–20.0)	static	Rexrode, 1987
Pimphales promeles (juveniles)	TBTO	96-h LC50 = 2.6 µg/L (2.3–2.9)	flow through	Brooke *et al.,* 1986
Fundulus heteroclitus, (subadults)	TBTCl	96-h LC50 = 23.4 µg/L (15.2–30.9)	flow through	Bushong *et al.,* 1988
Fundulus heteroclitus (adults)	TBTO	96-h LC50 = 24.0 µg/L	static	Rexrode, 1987
Fundulus heteroclitus (adults)	TBTO	96-h LC50 = 17.2µg/L	flow through	Pinkney *et al.,* 1989
Cyprinodon variegatus (subadults)	TBTCl	96-h LC50 = 25.9 µg/L (22.8–30.1)	flow through	Bushong *et al.,* 1988

Table 2.5 Acute toxicity values of tributyltin compounds to various aquatic animals (Continued)

Organisms	Tributyltin compounds	Effect (Confidence limit)	Types of exposure	Reference
Brevoortia tyrannus (juveniles)	TBTCl	96-h LC50 = 4.5 µg/L (3.6-6.4)	flow through	Bushong *et al.*, 1988
Menidia menidia (adults)	TBTCl	96-h LC50 = 8.9 µg/L (6.7-11.6)	flow through	Bushong *et al.*, 1988
Menidia beryllina (larvae)	TBTCl	72-h LC50 = 4.6 µg/L	flow through	Bushong *et al.*, 1987
Menidia beryllina (adults)	TBTCl	96-h LC50 = 3.0 µg/L (2.3-4.0)	flow through	Bushong *et al.*, 1988
Salvelinus namaycush (subadults)	TBTO	96-h LC50 = 5.21 µg/L	flow through	Martin, 1989
Oncorhynchus tshawytscha (juveniles)	TBTO	96-h LC50 = 1.5 µg/L	static renewal	Short&Thrower, 1986
Tilapia rendalli (subadults)	TBTO	24-h LC50 = 53.2 µg/L	flow through	Chliamovitch and Kuhn, 1977
Lepomis macrochirus (juveniles)	TBTO	96-h LC50 = 7.6 µg/L (5.6-10)	static	Rexrode, 1987

Table 2.6 Chronic toxicity of tributyltin compounds to various aquatic animals

Organisms	Tributyltin compounds (Concentrations)	Effects	Types of exposure	Reference
Gastropods				
Nucella lapillus	TBT as leachate (1, 17 ng/L)	Imposex observed after exposure to TBT over a period of 12 weeks.	flow through	Gibbs *et al.*, 1991
	TBTO as leachate (3.6,18.7,107 µg/L)	Imposex were observed. Rproductive failure and concomitant lack of recruitment has led to the decline of populations.	static	Gibbs&Bryan, 1987
Bivalves				
Mytilus edulis	TBTO (0, 0.02,0.064, 0.5, 1.0, 10 µg/L)	General trends to decrease growth rate when exposed to TBT above 0.5 µg/L.	flow through for 60 days	Guolan& Young, 1995
	TBTO (0.1, 0.4, 1.0, 2.5, 5.0, and 10.0 µg/L)	Significant reductions in growth rate (length) occured at ≥ 0.4 µg/L.	flow through for 7 days	
	TBT as leachate	Reduced growth rate at concentration of 0.24 µg/L.	flow through for 45 days	Thain, 1986
Ostrea edulis	TBT as leachate	Larval production was inhibited at 0.24 and 2.6 µg/L.	flow through for 75 days	Thain, 1986

Table 2.6 Chronic toxicity of tributyltin compounds to various aquatic animals (Continued)

Organisms	Tributyltin compounds (Concentrations)	Effects	Types of exposure	Reference
O. edulis	TBTCl	Rate of oxygen uptake increased two-fold when TBT concentration was increased from 0.5 to 10 µg/L. Feeding rate was significant reduced above a threshold concentration of 3 to 4 µg/L. A severe inhibition of growth at above 4 µg/L.	flow through	Widdows & Page, 1993
Crassostrea gigas	TBT as leachate	Significantly reduced growth rate at a concentration of 0.24 µg/L.	flow through for 45 days	Thain, 1986
C. gigas	TBTO (0.01, 0.02, 0.05, 0.1, and 0.2 µg/L)	Significant effects were found as little as the of 0.05 µg/L for oxygen consumption and feeding rate, 0.02 µg/L for growth and 0.01 µg/L for compensation for hypoxia.	static renewal	Lawler & Aldrich, 1987
Villorita cyprenoides	TBTO (0.006, 0.008, and 0.01 mg/L)	Pronounced decline in the oxygen consumption of the clams. TBTO exposed animals consumed well below one-tenth of the oxygen consumed by the control animals.	static renewal	Sujatha *et al.*, 1996

Table 2.6 Chronic toxicity of tributyltin compounds to various aquatic animals (Continued)

Organisms	Tributyltin compounds (Concentrations)	Effects	Types of exposure	Reference
Venerupis decussata	TBT as leachate	Reduced growth rate at a concentration of 0.24 µg/L.	flow through for 45 days	Thain, 1986
Mercenaria mercenaria (larval, juveniles)	TBTO (0-10 µg/L)	Valve length of veligers was statistically less than controls at the concentration of 50 ng/L. TBT clearly exhibited its effects on larval development.	static renewal and flow through	Laughlin Jr., 1986
	TBTO (control, 0.6, 1.0, 2.5, 5.0, 7.5 µg/L)	Growth of veligers was distinctly reduced when exposed to TBT for 8 days.	static renewal	Laughlin Jr. *et al.*, 1989.
Crustaceans *Daphnia magna* (adults)	TBTO (control, 0.1, 0.2, 0.5, 1.0, 2.1 µg/L)	The number of young produced per adult serving and the number of young produced per adult per reproductive day were significantly reduced at concentrations ≥ 0.2 µg/L	static renewal (21 days)	Brooke *et al.*, 1986
	TBTCl	At concentrations below which cause mortality (approximately 0.5 µg/L), individuals exhibited abnormal photobehaviour, incessantly swimm with a high rate of antennal strokes against the container wall that was closet to the light.	static renewal	Meador, 1986

Table 2.6 Chronic toxicity of tributyltin compounds to various aquatic animals (Continued)

Organisms	Tributyltin compounds (Concentrations)	Effects	Types of exposure	Reference
Uca pugilator (adults)	TBTO (0-50 µg/L)	Retardation of limb regeneration and eodysis after exposure to 0.5 µg/L.	Static renewal	Weis *et al.*, 1987
Acanthomysis sculpta	TBTO as leachate 0.03-0.52 µg/L	Inhibit growth and development of juveniles and subadult at levels of 0.4 and 0.5 µg/L. Reproductive effects were reported at 0.33 and 0.19 µg/L. No observe effect level (NOEL) was 0.09 µg/L.	flow through for 65 days	Davidson *et al.*,1986.
Gammarus sp.	TBT (0, 29, 49, 120, 223, 579 ng/L)	Weight of the animals exposed to control conditions (0 µg/L) was 2.8 times greater than those exposed to 0.579 µg/L.	static renewal	Hall *et al.* 1988
<u>Fishes</u> *Fundulus heteroclitus*	TBT (0-30 µg/L)	Delay and inhibition of hatching,and showed teratological effects of 30 µg/L.	static renewal	Weis *et al.*, 1987
Pimephales promelas (eggs)	TBTO (0, 0.02, 0.08, 0.15, 0.45, 0.92, 2.20 µg/L)	At exposure of 0.45 µg/L and above, mean fish weight was significantly less than that of the control. Mean standard lenght was significantly reduced at the lowest exposure(0.08µg/L). NOEL was between 0 - 0.08µg/L.	flow through for 33 days	Brooke *et al.*, 1986

Table 2.6 Chronic toxicity of tributyltin compounds to various aquatic animals (Continued)

Organisms	Tributyltin compounds (Concentrations)	Effects	Types of exposure	Reference
Salmo gairdneri	TBTO	At concentrations from 5.85 to 0.0117 mg/L resulted in damage to gill epithelium. At lower concentrations, a degradation of the cornea and damage to the epithelial cells of bile ducts were also observed. With concentrations from 0.023 to 1.17 mg/L, TBTO interfered the respiration process.	flow through	Chliamovitch & Kuhn, 1977
Menidia beryllina (adult)	TBT (0, 93, 490 ng/L)	Significant reduction in growth in 93 and 490 ng/L.	static renewal	Hall *et al.*, 1988

Note : TBTCl = Tributyltin chloride   TBTO = Tributyltin oxide   TBT = Tributyltin

Sea bass (*Lates calcalifer*)

Taxonomy

Phylum   Vertebrata
Subphylum   Craniata
Superclass   Gnathostomata
Series   Pisces
Class   Teleostomi
Subclass   Actinopterygii
Order   Perciformes
Suborder   Percoidei
Superfamily   Percoidae
Family   Centropomidae
Genus   *Lates*
Species   *Lates calcalifer*

Common names: white sea bass, sea bass, sea perch, giant perch, cock-up, Koro, Saihap, barramundi

Thai common name : pla kaphong, pla kaphong khao, or pla kaphong namjerd

Characteristics and biology

Sea bass or the , so called, giant sea perch is one of the large brackishwater fish found mainly in estuaries and mangrove swamps adjacent to the sea. Sea bass is one of the economically important fishes, widely distributed along the coast of the Gulf of Thailand and the Andaman sea. It is also found in the Indo-Pacific and Australian regions. *L. calcalifer* is the largest fish comparing to other members of the same genera.

The body form of the sea bass is oblong and somewhat compressed. The head is depressed, the upper profile being slightly concave. The cleft of the mouth is slightly oblique. The maxilla extends to below the posterior edge of the orbit. Villiform teeth appear on jaws, vomer, and palatine bone. The opercles are strongly denticulated. Two dorsal fins unite at their bases, the first with seven or eight spines and the second with ten to eleven soft rays. The anal fin has three spines. The caudal fin is rounded and fan-shaped. The scales are ctenoid, and of moderate size. There are 52-61 scales on the lateral line.

Sea bass fry at approximately 0.5-1.0 cm in length are uniformly dark brown with scattered yellow spots. The bigger fry (2.0 cm long) exhibit distinct markings. A brown band runs on the upper part of the body from the end of the mouth to the dorsal fin, the side of the body is yellowish-brown with four to five dark grey stripes. These markings will disappear as the fish grows.

L. calcalifer is widely distributed in marine, brackish and fresh waters which connect to the sea. The fish finds shelter and food in littoral waters and prefer slow moving clear water. It has a wide salinity tolerance and can be acclimatized to freshwater. It is extremely predacious (mainly other fish, shrimps, snails, and worms). Cannibalistic effect is known when food is limited. Spawning takes place during the early rainy season (May-September) in areas of low salinity, muddy bottom and dense growth of mangroves. The eggs and larvae are carried upstream by the tide into mangrove swamps which serves as nursery grounds. (Southeast Asian Fisheries Development Center Training Department, 1995 ,and Australian Center for International Research, 1986)

The sea bass (*L. calcalifer*) was selected in this study because :
1. Widely available and abundant in Thailand
2. Represent of the ecosystem that may receive the impact
3. Commercial and ecological importance
4. Ameanable to routine maintenance and available techniques for culturing and rearing in the laboratory
5. Adequate background information