Chapter 2

Literature Reviews

2.1 Background

Bis-tributyltin oxide is for simplicity usually referred to as "tributyltin oxide" or generally known as the acronym "TBTO". This chemical is one of several tributyltin compounds each of which exhibiting different physical and chemical properties, for example tributyltin fluoride, tributyltinmethacrylate copolymers, tributyltin naphthenate, etc. TBTO is itself used by the chemical and paint industries for the manufacture of other ingredients in the formulation of antifouling paints and industrial wood preservative properties of relatively low percentage levels.

Tributyltin oxide is the major derivative produced commercially from the starting material tributyltin chloride. By aqueous saponification of the chloride, sodium chloride is the by-product and removed from the process in aqueous layer separation (for simplicity the butyl radical, C_4H_9, is shown as "Bu").

$$2BUSnCl + 2NaOH \rightarrow Bu_3Sn\text{-}O\text{-}SNBu_3 + 2NaCl + H_2O$$

Tributyltin oxide is colourless liquid of molecular weight 596 with a faint typical odour. Its solubility in water is low, varying between 0 and 10 mg/l according to pH, temperature and salinity. TBTO is soluble in most organic solvents. Commercial grades of TBTO are produced to a 95/97% specification. The impurities present are tetrabutyltin, added stabilizer and dibutyltin derivatives. Higher-grade purities are produced by vacuum rectification. The tin metal content is just under 40%.

As well as other butyltin compounds, TBTO is predominantly used as active ingredients in the formulation of antifouling paints and industrial wood preservations of relatively low percentage levels. Antifouling paint is applied to the hulls of vessels to prevent fouling, the growth of organisms such as barnacles, shells, weed, and algae. Fouling organisms have the ability to attach themselves quickly and firmly on ships' hull with the capacity for very rapid initial growth and a vast reproductive potential. Hundred tons of fouling can be accumulated in period less than a year. Fouling occurs on the hulls of ships and boats, causes drag and slows

vessels speeds due to increase friction between the hulls and seawater. These drags together with added weight of the fouling resulting in considerable increased fuel consumption and a loss of maneuverability. There are varieties of fouling organisms, several hundred species of those have been identified on the fouled hulls of ships. These include diatoms, algae (green, brown and red weeds), as well as various solitary organisms like hydroids, tubeworms, and bryozoa.

Historically, a wide range of active chemicals is applied as biocide in the past to combat fouling—pitch, lead, tin, and copper sheeting. Tar and bitumen were in widely used in the eighteenth centuries. A century ago copper-rosin based antifoulants were introduced, and improved for better effectiveness and life expectancy during the 1950s, newly available toxicants were added as "booster". Such products as DDT, phenylmercury, organolead, and organic compounds were used as booster biocide. By 1960 triorganotins were employed in copper based antifoulants, TBTO and TBT sulfide were used as main biocide in alkyl, vinyl and acrylic substrate systems of the original formula. Organo-lead and organo-mercury were banned because of relatively high toxicity and adverse environmental effects. The spray painting technique was introduced in the 1970s, arsenic additive was finally withdrawn from use. Its antifouling performance was tested along the East Coast of America and Gulf of Mexico, and showed their effectiveness. Furthermore, Unlikely the traditional copper based red/brown coating, TBTO has ability to be present in a wide range of brightest colours. Triorganotin based antifouling paints were soon applied for commercial vessels.

In addition to use TBTO as booster in antifoulants, it was also applied in wood preservation. Due to the very effective fungicidal and bactericidal activities of TBTO, It shows good effects against Gram-negative bacteria and extremely against Gram-positive species. After wide spread use of TBTO based preservative products by amateur or domestic application declined, TBTO and TBT naphthenate based formulations are approved to be used only in industrial and for a limit number of professional applications. Originally, TBTO and TBTN based wood preservatives were formulated in aqueous forms. The wood has immersed in an open vessel for a few minutes, removed and allowed draining and drying. Unfortunately, it causes distortion in joinery products if swelling takes place, and consumes long drying time. Because of unpreferrable disadvantage in industrial application, the formulation in light organic solvents were employed and applied in closed systems with solvent and preservative recovery.

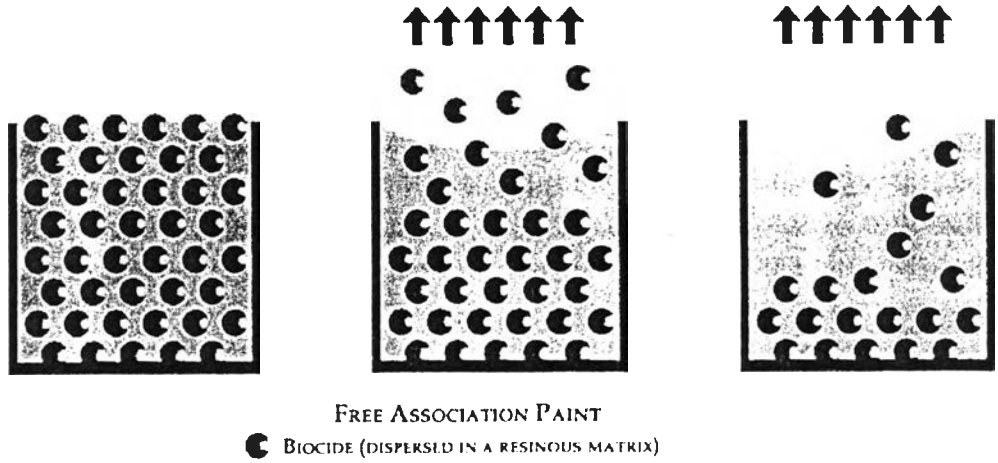
2.2 Types of tributyltin paints

TBTO and other derivatives especially triorganotin-based antifouling paints were worldwide applied because of their high microbiological and fungicidal toxicity and comparably low mammalian toxicity. Those paints can be divided into 2 systems according to the release mechanisms: free association and copolymer systems.

2.2.1 Tributyltin free association system

Historically, conventional anti-fouling paints, which have been in use since the last century, are based on a soluble matrix. This type of anti-foulant is sometimes referred to as "free association". The active ingredients are physically mixed to disperse in a rasinous matrix called "rosin" and from which they can slowly leach in seawater. Rosin is a natural product obtained from trees, mainly consisting of a mixture of acids. This type of paint system has been the basis of the most traditional anti-foulants used for century. The rate of release of the biocides is uncontrollable, at unpredictable coating lifetime. These features depend upon quality of the products and the thickness of application as paint. It can be exhausted in a short period, by one to two years. Biocides are initially release very rapid from the matrix, subsequently declined in leaching level.

To maintain the performance of the anti-fouling paint, the biocide in insoluble matrix has been developed during late 1960s. Chlorinated rubber and vinyl resin were employed for this formulation as chemical base resulting in longer lifetime by a period of two years because of their higher mechanical strength. The release rate of biocide in insoluble based anti-foulant shows similar trend of soluble based, initially high leaching and exponentially declined dissolution with time. The operational mechanism of the free association paint is illustrated in Fig. 2.1



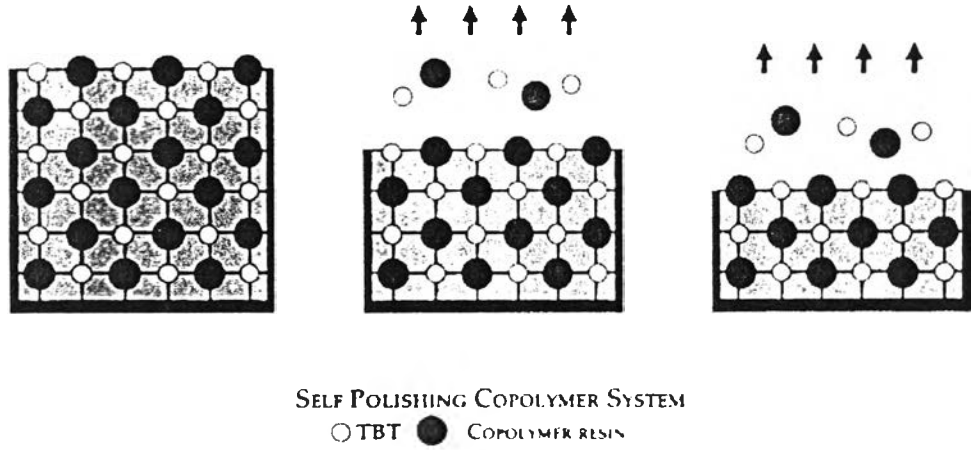
The biocide leaches freshly from the resinous matrix. The initial release is rapid and uncontrolled. Subsequent release declines steadily from the matrix such that the anti-fouling performance of the paint diminishes with time.

Fig. 2.1 Operational mechanism of a free association paint. (modified from Bennett, 1996).

2.2.2 TBT self polishing copolymer system

TBT self polishing copolymer (SPC) paints are currently used. This system is a combination of biologically active resins and other active ingredients, typically TBT copolymer resins and copper compounds. A TBT biocide is chemically bonded uniformly within and throughout a copolymer resin system. The coating on a ship that has been painted with TBT-SPC paint system reacts by hydrolysis with seawater, resulting in sloe and uniform release of tributyltin oxide, which combat fouling. TBT biocide erosion only takes place at the surface of the coating, in the top few nanometer surfaces. The remaining surface that TBT already leached away is mechanically weak and is eroded by moving seawater. This repeated reaction occurs throughout the lifetime of coating, then allows new surface to expose to the seawater, and keeps anti-fouling performance until the coating is exhausted. Generally, this lifetime of these systems can exceed five years.

Fig. 2.2 illustrates the operational mechanism of a TBT self-polishing copolymer system in diagrammatic form.



At the point surface sea water hydrolyses the TBT copolymer bond and the TBT biocide and copolymer resin is slowly released at a controlled rate. A uniform anti-fouling performance is achieved throughout the life of the paint.

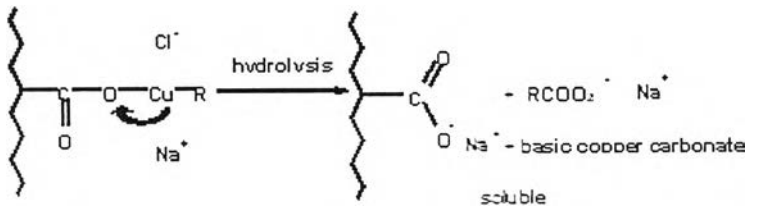
Fig. 2.2 Operational mechanism of a TBT self-polishing copolymer paint. (modified from Bennett, 1996).

2.3 Tin-free anti-fouling paints

Continuing endeavour since the late 1980s to search some new choices, tin-free anti-foulants, less harmful side effects and potential to accumulate in aquatic environment is in progress. Unfortunately, these efforts did not reach the goal although many alternative products were introduced.

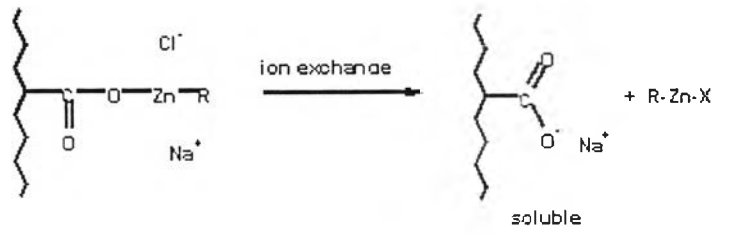
Copper-based system is an alternative in use, due to its limited range of effects, other booster biocides are essential to incorporate in such formulae for wide spectrum activities. Irgarol 1051 is a herbicidal additive employed in copper-based anti-fouling paints (Stewart, 1996). Operational mechanisms of some tin-free anti-foulants, which have recently been introduced, are illustrated in Fig. 2.3. Current organic and metal-based anti-fouling biocides are summarized in Table 2.1, possible hazards of which is shown in Table 2.2. The feature of TBT SPC versus tin-free system is compared in Table 2.3.

Copper-acrylates



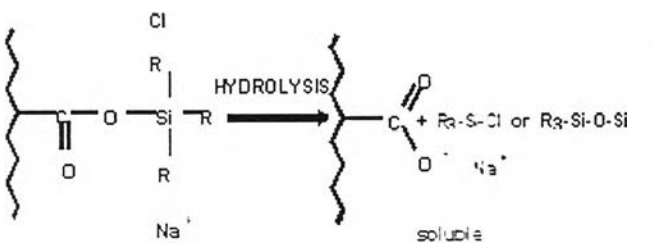
Nippon paint: Ecoloflex® SPCI
International Paint: Intersmooth Ecoloflex®

Ionexchange copolymer



Kansai Paint: Exion®

Silane functionalized methacrylates



R = alkyl, aryl; e.g. I-propyl, butyl
Si = silicone
Chugoku Marine Paint: Sea Grand Prix®
NOF: Takara Quantum®

Fig. 2.3 Operational mechanisms of some tin-free anti-fouling paints. (Modified from ORTEPA, 2001a)

Table 2.1 Organic and metal-based anti-fouling biocides.

Organic Biocides	Metal-based Biocides
Sea-Nine® 211 (dichloro-isothiazolone)	Copper(I) oxide
Irgarol® 1051 (triazine)	Cuprous thiocyanate
Diuron (dichlorophenyl-dimethyl urea)	Copper metal
Nopcocide/Chlorothalonil (tetrachoro isophthalonitril)	Copper resinate
Preventol A4/Dichlofluanid (dichlorofluoromethylthio-dimethylphenylsulfamide)	Copper naphthenate
Densil (tetrachloro-methylsulphonyl-pyridine)	Copper/Zinc pyrithione
Pyridinetriphenylborane (used in Japan only)	Zinc oxide
Thiram (thiocarbamate)	Zinc metal
	Zinc naphthenate
	Zineb (zinc carbamate)
	Mancozeb (manganese/zinc carbamate complex)
	Maneb (manganese carbamate)

Source: ORTEPA, 2001b

Table 2.2 Alternative anti-fouling biocides and additives, and their possible hazards

Active ingredient	Possible hazards
Copper	The use of copper as an anti-fouling biocide is historic and ubiquitous. Elevated levels of copper in sediments and biota have been associated with high density of pleasure boats and poor water exchange; but there are few, if any, reports of copper-induced environmental degradation. Copper speciation, and hence toxicity, is governed by pH and salinity. In freshwater lakes, particularly of low pH, copper toxicity may be concern.
Tetracycline	Tetracycline, an antibiotic, has been advocated as an additive to anti-fouling paint. It rapidly leached and degraded in sea water, and is unlikely to be either an effective anti-fouling biocide or a marine environmental hazard.
Triazine	The Triazine Irgarol 1051 is a herbicidal additive in some anti-fouling preparations. In a survey of coastal Mediterranean seawater, 16 out of 20 samples were contaminated with Irgarol 1051. Levels were highly variable, ranging up to 1700 ng l^{-1}, and were obviously related to boating activity. The environmental impact of these levels is unknown due to a lack of knowledge about sublethal effects of Irgarol 1051. Irgarol 1051 was also detected in all coastal estuarine and marina (but not riverine) samples in a survey of southern England coastal waters. Highest levels were found in marinas. Sediments were also found to be contaminated with Irgarol 1051, suggesting that partitioning onto particulate matter may be an important influence on the compound's fate. In contrast, low levels of the agriculturally derived triazine herbicides simazine and atrazine were found in river and estuarine water.

Source: modified from Stewart, (1996).

Table 2.3 Comparison of Tributyltin Self-Polishing Copolymer (TBT SPC) to Tin-Free Anti-fouling Paints (From ORTEPA, 2001c)

Issue	TBT SPC	Copper Acrylates	Silane Methacrylates	Ion Exchange Copolymers	Copper Ablatives	Other Combination Technologies
Extant Environmental Monitoring Data	Extensive (ongoing since 1980s)	No published data	No published data	No published data	Limited published data	No published data
Extant Aquatic Toxicity Data	Acute and chronic data available	No published data	No published data	No published data	Limited acute data available	No published data
Chronic Risk Assessment	Yes	No	No	No	No	No
Volatile Organic Compounds (VOCs)	Less than 30%	32-33%	Less than 30%	No published data	Less than 30%	No published data
Worker Exposure Study	Studies demonstrate no risks to properly equipped workers	No published data	No published data	No published data	No published data	No published data
Booster Biocide	Cu_2O (lower than 15%)	Cu_2O (40%) Cu-pyrithione (5%)	Cu_2O (40-53%) Cu/zinc-pyrithione (3-6%)	No published data	Cu_2O (ca. 40%) and others (triazine, isothiazolone, zinc-pyrithione, etc.)	triazine, isothiazolone, zinc-pyrithione, etc.
Contains Leachable Chlorinated Plasticizer	No	Yes	Yes (in some formulas)	No published data	Yes	No published data
Proven Dry-Docking Interval	Data for over 60 months are available	Manufacturer reports data for 36 months are available	No published data	No published data	Data for 30-36 months are available	Manufacturer reports data for 6-15 months are available

Table 2.3 cont.

Issue	TBT SPC	Copper Acrylates	Silane Methacrylates	Ion Exchange Copolymers	Copper Ablatives	Other Combination Technologies
Antifouling Performance	96% of vessels return with satisfactory performance after 60 months	Paint manufacturer reports 90% satisfactory performance after 36 months	No published data	No published data	70-74% satisfactory performance after 30-36 months	No published data
Cost Comparison	Not applicable	2 to 2.5 times the cost of TBT SPC[1]	No published data	No published data	2.5 to 4 times the cost of TBT SPC[1]	No published data
Paint Shelf Life	Longer than 12 months	2-3 months (temperature dependent)	No published data	No published data	No published data	No published data
Film Integrity	Excellent film properties, no cracking or detachment	Excellent film properties, no cracking	No published data	No published data	Poor film properties, prone to cracking and detachment	No published data
Overcoating Properties	Proven satisfactory results after high pressure freshwater surface preparation	Manufacturer reports satisfactory results after high pressure freshwater surface preparation	No published data	No published data	Expensive and time consuming surface preparation, slow drying times	No published data
Leaching Properties	Excellent; very thin active zone 10-90 nm; effective under static conditions	Fair; active zone 15000-25000 nm; no available data under static conditions	No published data	No published data	Ineffective; thick, mechanically weak, insoluble, inhibits biocide release; ineffective under static conditions	No published data

2.4 Tributyltin in fresh water environments

2.4.1 Sources and occurrence

Municipal wastewater is considered to be a significant source of TBT for rivers and other surface waters due to its considerably content occurrences (Schebek, Andreae and Tobschall, 1991; Fent and Müller, 1991; Chau, Zhang and Mcguire, 1992; Donard, Quevauviller and Bruchet, 1993). In addition, use of TBT in unregulated anti-fouling paints, in timber preservation, and in industrial applications as biocides or catalysts, are other contaminated source of TBT to rivers. Furthermore, effluents from a public wash-down yard, where boat scrubbing and high-pressure hosing are used to removed old layer of paint, also wastewater from a shipyard are highly contaminated by TBT at the concentrations of 26.1 and 12.2 $\mu g\ l^{-1}$, respectively. These waters thus, show high risk to contaminate into freshwaters without treatment due to the three order of magnitudes contents. Eventhough sediments act as a sink and reservoir, but also as potential sources of these compounds in reducing direct input (Fent and Hunn, 1995).

The occurrence of tributyltin is far fewer data for freshwater than seawater. Maguire (1996) summarizes such records of TBT in freshwaters environments and the highest content found in a harbour in Antwerp, Belgium is 7,300 ng Sn l^{-1} before banning. After organotin containing anti-foulant regulation has been introduced worldwide, TBT concentrations in habour water, for example, is between 40 and 50 ngl^{-1} in 1993 post-banning in Switzerland.

2.4.2 Persistent and fate

In case of habour waters, TBT appeared to be present predominantly in operationally defined "dissolved" phase, i.e., that passed a 0.45 μm filters (Fent and Hunn, 1991). Otherwise, the adsorption process and partitioning to particulate matter with subsequent sedimentation is a significant transport route of TBT in lakes. This resulting in a significant accumulation of this compound in the sediment. In addition to sedimentation of suspended particulates, bioaccumulation is nother transfer pathway of TBT, in particular by mussels. High TBT residues in the range of 0.7 to 2.1 $\mu g\ g^{-1}$ (wet weight) were determined in 1993. A generalized scheme of the distribution of TBT in different compartments in freshwater habours based on analytical determinations in Swiss river systems shows the highest content of about 1.1-9.4 x 10^3 $\mu g\ kg^{-1}$ wet

weight in mussels. Decreasing orders of TBT concentrations are found in particulates, sediment and water in the ranges of 1.0-2.0 x 10^3, 0.04-0.7 x 10^3 $\mu g\ g^{-1}$ and 0.1-0.8 $\mu g\ l^{-1}$, respectively (Fent and Hunn, 1995).

The fate of TBT is the result of simultaneous and combined process and transfer paths. The key processes include mixing and dilution into surroundings, biodegradation, adsorption to suspended particulate, and subsequent scavenging to sediments. In the sediments, TBT is persistent, and the degradation is only minor (Fent and Hunn, 1995). Maguire (1996) summarized the persistence of tributyltin in freshwater environments. It manifests that TBT is only low-to-moderate persistence in freshwater (half-lives of several days to a few months), depending upon various factors such as the presence or absence of tributyltin-degrading microorganisms, temperature, the degree of insolation, and the season of the year. Nevertheless, TBT appears to be at least moderately persistent in sediment, with measured half-lives in the order of months and estimated half-lives from sediment core data in the order of years.

2.5 Tributyltin in estuarine environments

2.5.1 Sources and concentrations

In estuaries and habours, large vessels are revealed to be the major source of TBT. In case of small estuaries, marinas and moorings for small pleasure craft can contribute significantly to a dissolved TBT load as well. The concentration of TBT found in surface water and sediment was closely related to the activities of ship in particular area. As in vicinity of the dock area of Tianjin New Harbour Shipyard for example, TBT in water, pore water and sediment were ranged from 15.69 to 41.78. 20.96 to 53.48 ng l^{-1} and 26.4 to 62 ng g^{-1}, respectively (Ma, Dai and Huang, 2000).

Levels of TBT in waters, sediments and biota have not revealed the same decreased trend as in marine environments worldwide. The major continuing source manifests to be dry-docks and other facilities where ship building, repair and repainting occurs, as the area affected by traffic of larger vessels show relatively low TBT concentration. Tributyltin concentrations in some estuarine environments are summarized by Batley (1996).

2.5.2 *Distribution and fate*

In natural waters, TBT is present predominately as neutral TBT-OH or as cationic TBT^+ species (Arnold *et al.*, 1997). The distribution of TBT in estuarine environments after releasing from sources was determined by partitioning (into pore water) and sorption (including adsorption and desorption) processes.

Partitioning process

Based on the study of Ma *et al.*(2000), TBT first partitioned into pore water and reached the maximum concentration rapidly due to the limited capacity. Meanwhile, TBT in the pore water was quickly distributed to the sediment and resulting in rapid decreasing of TBT concentration because the sediment had high binding ability. Finally, the equilibrium among overlying water, pore water and sediment was achieved.

The rate of TBT partitioning into the pore water was more rapid than that of TBT sorption on the sediment. The salinity had a significant effect on TBT partitioning into the pore water (Fig. 2.4). For example, the partitioning coefficient between pore water and overlying water (expressed as the ratio of TBT concentration in the pore water to that in the overlying water) at 35‰ salinity was less than one-half of the value at 5‰ salinity. (Ma *et al.*, 2000) Partitioning of TBT between the sediment aqueous phase is influent by total organic matter in sediment (Lanston and Pope, 1995). The binding sites of dissolved organic carbon (DOC) on which released organic matter coated sediment could promote the partitioning of TBT into pore water (Fent and Looser, 1995). In case of salinity increased, DOC would be flocculated (Romkens and dolfing, 1998). Consequently, the binding site of remaining DOC would predominately occupied by Ca^{2+} and Mg^{2+} due to their much higher concentration. As the result of increasing salinity, TBT partitioning into the pore water would be subsequently decreased. For pH, the effect on partitioning of TBT into pore water appeared to be a positive relationship at pH < 7.5. It said that the partitioning coefficient (C_p/C_w) increased with increasing pH. It reached a peak at pH 7.5 and then decreased until pH 8.0 (Fig. 2.5). After that, pH had little or no effect on the partitioning coefficient at pH > 8.0 (Ma *et al*, 2000).

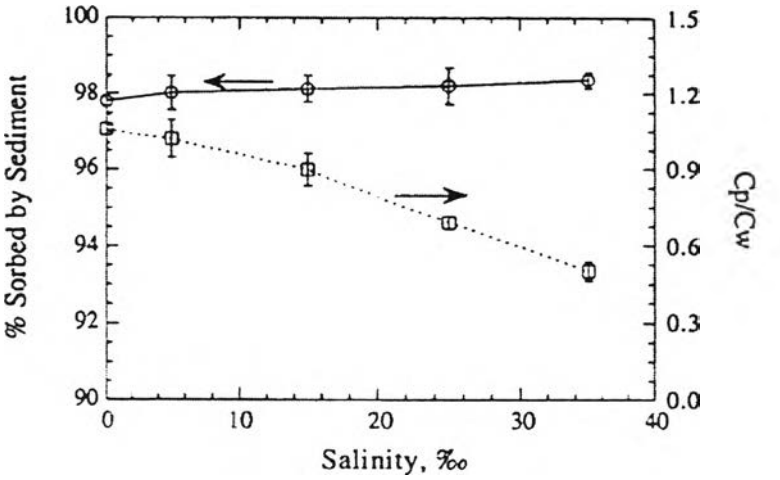


Fig. 2.4 Salinity effects on the sorption of TBT by the sediment and on the partitioning of TBT into the porewater, pH = 7.90 and 5% formaldehyde was used. (From Ma *et al.*, 2000)

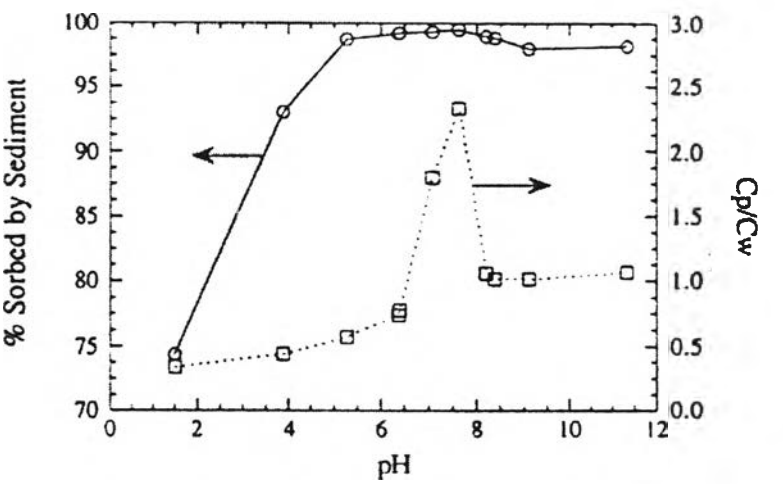


Fig 2.5 pH effects on the sorbtion of TBT by the sediment and on the partitioning of TBT into pore water. Salinity = 15 ‰ and 5% formaldehyde was used. (From Ma *et al.*, 2000)

Sorption processes

For sorption reactions, the rate of adsorption and desorption were fastest during the first 30 min with 98% of the maximum adsorbed amount by the sediment and 0% of the equilibrium aqueous TBT concentration released from sediment were respectively achieved. After that, the rate decreased gradually until the equilibrium was reached within 6 h. A heterogeneous phase adsorption kinetic model by Kue and Loke was applied to describe the kinetic results of TBT adsorption to the sediment. The model can be expressed mathematically:

$$k = \frac{S}{C_w t^{\frac{1}{m}}}$$

where k is the mass transfer coefficient; S is sorbed TBT amount by the sediment in μg kg^{-1}; t is time in min; and m is adsorption constant.

Sorption process follows biphasic kinetics, which characterizes by rapid reaction rate followed by a much slower reaction rate. For adsorption process, TBT distribute rapidly to the external sites which is readily accessible, and then transfer to the poorly-accessible internal sites with much slower rate. In case of desorption, the release of TBT from external site is quick, whereas from internal site is slow.

According to the study of Ma *et al.* (2000), salinity ranging from 0 to 35‰ had little effect on he sorption of TBT on the sediment because the hydrophobic partitioning mechanism predominated at the experimental pH of 7.90. On the contrary, pH had strong influence on both sorption of TBT on the sediment. The adsorption on the sediment was decreased with decreasing pH due to the competition of other cations with TBT^+ if pH was below the pK_a of TBT. While at pH values around and greater than pK_a, pH had little effect on the adsorption. The sorption processes of TBT on sediment are reversible. Sediment can serve as a sink for TBT and TBT contaminated sediments may act as sources for dissolved TBT as well.

In addition, composition of sediment is also a factor on which affects the sorption processes. For instance, more TBT was desorped from the sandy sediment into pore water and overlying water than from smaller grained, muddy sediment (Austin and McEvoy, 1995). By considering the partition coefficients between sediment and water equilibrium, or K_d values, that are an order of magnitude lower for open coastal beach sandy sediments than for mud estuarine sediments (Lanston and Pope, 1995).

2.5.3 Degradation processes

It has been revealed that the degradation of TBT principally takes place in sediments by microorganisms (Dowson, Bubb and Lester, 1996). After TBT in overlying water partitions rapidly into pore water. Microbes could promote the partitioning of TBT in different layers of pore water and sediment. Microorganisms in pore water or on sediment might use TBT as their carbon energy source and degrade TBT to dibutyltin and monobutyltin to the end product of inorganic tin.

The above idea is supported by the study of Ma *et al.* (2000) that TBT concentrations in overlying water, different layers of pore water and sediment were compared between septic and aseptic systems including the kinetics. It found that the aseptic system took much more time to reach the maximum concentration of TBT in pore water, 8 days versus 2 days for septic system. The equilibrium concentrations were achieved at around 14 days in the aseptic system while 4 days was obtained in the septic system.

The TBT in the septic system was better distributed in different layers of pore water than that in the aseptic system., 53-83% of total TBT concentration partitioned in all 0-4 cm pore water was found in 0-1 cm pore water, followed by 14-42% and 4-26% in 1-2 cm and 2-4 cm pore water, respectively. On the other hand, most of TBT up to 96% was determined in the first 0-1 cm pore water of aseptic system, TBT fractions in 1-2 cm and 2-4 cm pore water were very small.

The adsorption of TBT on sediment in both aseptic and septic systems was predominately on surface 0-1 cm sediment; 99.5% for the former and 90-98.7% for the later. In addition, the first 1 cm layer sediment reached the peak of adsorption by 2 days earlier the 1-2 and 2-4 cm layers.

In deeper, anoxic sediment, the persistence of TBT was considerably longer than surface sediment, which may be related to the greater stability of TBT-sulfide.

TBT degradation products, DBT, MBT, and even inorganic tin were detected in the lower layer of some sediment samples in the septic system. There are large differences between TBT concentrations in the aseptic and septic systems, especially the upper 0-1 cm layer water and sediment.

In addition, Volatilization is a degradation pathway of TBT although it is considered to be minor and not important. Biological and/or chemical methylation mechanisms are likely to occur in sediments and to lead to remobilization of tin species into the water column and subsequently to the atmosphere (Amouroux, Tessier and Donard, 2000).

2.6 Recovery of TBT contamination after TBT registrations

After the TBT regulations were introduced worldwide, imposex in various gastropods was utilized to elevate the contaminated status by many countries. The degree of imposer as determined by the relative penis size index (RPSI), is a useful alternative for this. For instance, In assessment of TBT contamination was performed in England using the occurrence of imposex in the European dogwhelk *Nucella lapillus*, the severity has declined dramatically during the past decade. No imposer was presented or poorly developed at the large majority of sites in the North Atlantic, including coasts near major shipping lanes (Evans *et al.*, 1998). Another, a general recovery in dogwhelk, *N. lapillus*, populations from the impact of TBT at the majority of the study sites in the Firth of Forth, UK, was found (Miller, Fernandes and Read, 1999).

In New Zealand, imposex decline was observed after restrictions were introduced on the use of organotin anti-fouling paints. Percentage of imposer and RPSI in female dogwhelk *Lepsiella scobina* was significantly reduced in area where subject only to pleasure craft. While a significant decline in the RPSI was observed in a habour area where subject to both pleasure craft and commercial vessels (Smith, 1996).

In the North Sea and Clyde Sea, the severity of imposex induced by TBT was declined after the introduction of regulations prohibiting the use of TBT-based anti-foulants on vessels less than 25 m in length. Extinction of *N. lapillus* was locally found due to earlier high levels of contamination, while the Relative Penis Size Indices (RPSI) of imposex have declined dramatically in survivors. However, centers of commercial shipping activity are still hot spot of TBT contamination (Evans, Evans and Leksono, 1996).

The recovery from TBT contamination after banning using female neogastropods as bioindicator, was evidenced in many areas of the Pacific Northwest especially the coast of Vancouver Island and in some locations in Strait of Georgia (Tester, Ellis and Thompson, 1996).

By contrast, in eastern Canada, imposex in *N. lapillus*, local bioindicator, still displayed and continuing concern despite the present of TBT restriction (Prouse and Ellis, 1997). As well as in France, where TBT was partially banned in 1982, showed little or no recovery from TBT contamination by using *Hinia incrassata*, *H. Reticulata* and *N. lapillus* as bioindicator (Oehlmann *et al.*, 1998). The determination of organotin compounds in 1997 around French coasts, indicated that the contamination was still problem. Seventy-five percent of the measurements were above the threshold of 1 ng l^{-1} which could cause toxic effects to marine organisms (Michel and Averty, 1999).

2.7 Ingestion, metabolism and bioaccumulation

The bioaccumulation of TBT is related to ingestive pathway of the organisms. In case of filter feeding clams *Venerupis decussata* collected from southwest Spain, the correlation between TBT bivalve contents and water concentrations were higher than those found between biota and sediments. The long-term trend of TBT in clam *V. decussata* indicated half-live values of about 7-14 years (Gómes-Ariza, Giráldez, and Moraldes, 2000).

In case of mammals, butyltin compounds were also detected in liver tissue of humans and raccoon dogs (*Nytoreutes procyonoida*) from Japan with concentrations of <360 ng g^{-1} wet wt. Takahashi *et al.* (1999) suggests that BTs in the form of plastic stabilizer and catalysts other than those of marine origin as anti-fouling agents, are alternative sources of exposure. Butyltin compounds containing in baking parchment could be transferred to foodstuff.

Butyltin compounds (BTs) were metabolized to dibutyltin (DBT), monobutyltin, and metalic tin as final product. BTs preferentially accumulated in liver or kidney compared to other parts of body, for example in bottlenose dolphin (*Tursiops truncatus*), bluefin tuna (*Thunnus thynnus thynnus*) and blue shake (*Prionace gluaca*) collected from Italian coast of the Mediterranean Sea in 1992-1993. The degradation product DBT was found in higher proportion in liver of dolphin and tuna while TBT was predominant in blubber of dolphin or muscle of tuna. For shark, TBT was predominant in all tissues, with the highest concentration in kidney. Accumulation of BTs in liver/kidney appears to be related to the presence of proteins such as glutathione (Kannan *et al.*, 1996).

TBT concentrations in different parts of common cormorants (*Phalacrocolax carbo*) from Lake Biwa, Japan were determined and showed increasing concentrations as the following orders: muscles, feathers, skin, liver, rest of tissues and organs. About oneforth of TBT body burden in feathers would be excreted during complete molting cycle, which has been a natural detoxification mechanism in these birds (Guruge *et al.*, 1996).

Spatially, ratios of TBT to total butyltins in fish from sea areas were higher than those from rivers. Eleven species of fishes from the port of Osaka and Yodo River, Japan were determined for TBT compounds. The results show that the ranges of 0.011-0.182 mg kg^{-1} wet wt was detected. The concentrations were not related to total length fish, and no correlation was also found between TBT and lipid content. Japanese seaperch was chosen to investigate TBT sex relationship, the results show that no difference was found (Harino, Fugushima and Kawai, 2000). Thje same result also found in river otter (*Lutra canadensis*), butyltin compounds were found in its liver at a range of 8.5-2610 ng g^{-1} wet wt. The significant greater concentrations were detected in river otters caught from area adjacent to major shipping activity, such as Puget Sound, Port Ward, Washington compared to those from rivers. The concentrations ranged from comparable (Puget Sound) to less (rivers) than what was found in coastal cetaceans (Kannan *et al.*, 1999).

In term of vertical distribution over the water column, the comparison of butyltin compound contents between deep sea (135-980 m in the aphotic bathyal zone) and shallow waters were conducted in Suruga Bay, Japan. Total butyltin (MBT+DBT+TBT) concentrations in the tissues of deep-sea fish, crustaceans, cephalopods, echinoderms, and gastropods were up to 980, 460, 460, 130, and 21 ng g^{-1} wet wt, respectively. These levels were lower than those in shallow water organisms. Among BTs, TBT was the predominant compound except in cephalopods (Takahashi, Tanabe and Kubodera, 1997).

Temporally, TBT levels found in the dogwhelk (*N. lapillus*) and the blue mussel (*Mytilus edulis*) from southwestern Iceland showed a seasonal fluctuation. In summer, concentrations were approximately five (dogwhelk) to ten (blue mussel) times the winter concentrations. The fluctuation was associated with seasonal activity in feeding and resting of the dogwhelk, and feeding of the blue mussel. while seasonal changes in shipping activity were insignificant (Skarphédinsdóttir *et al.*, 1996).

The range of 200 to 1100 ng g^{-1} wet wt as Sn of TBTs were detected in mollusks *Littorina littorina* and *N. lapillus* from the Catalan coast, Spain (Kure and Depledge, 1994). In clam, *Ruditapes decussata*, TBT accumulation was extremely rapid. It reached a maximum tissue concentration of 290 ng g^{-1} wet wt 3 weeks after exposure, which represent a bioaccumulation of 12000 (Morcillo and Porte, 2000). BTs (including tri-, bi- and monobutyltin) were detected in most of green mussels (*Perna viridis*) samples in Thai coastal waters, ranging from 4 to 800 ng g^{-1} wet wt. Bioconcentration factors (BCF) in Stenoglossan prosobranchs *Hinia incrassata* collected along the coast of Brittany and Normandy were between 46800 and 122000 (Oehlmann *et al.*, 1998).

As regards transferability to offsprings, the estimated BCF for TBT in *Crassostrea gigas* was 25000. Furthermore, 19% of total body burdens of TBT were found in gonadal mass prior to spawning period, indicating that a proportional amount of TBT would release with a following reproductive process (Shim *et al.*, 1998). Conversely, the estimated concentration ratio of butyltin compounds including TBT, DBT and MBT, in the liver of whale fetus to its pregnant mother was 0.015, indicative that transplacentral transfer of BTs from mother to her fetus is a deal less. Among the marine mammals collected from Japanese coastal waters, lower BT concentrations were found in pinnipeds compared with the cetaceans, suggestive of possible difference in degradation capacities and excretory moulting between these two groups of animals (Tanabe *et al.*, 1998).

In point of ecological scale, no biomagnification was detected through the food chain of Otsuchi Bay, Japan, but BTs may accumulate in biota at elevated trophic levels. Relatively high concentration of butyltin compounds were found in caprellid and smaller fish, such as gunnels (Table 2.4), with TBT as the predominant compound (Takahashi *et al.*, 1999).

Table 2.4 Butyltin concentrations (ng g^{-1} wet wt) in organisms collected worldwide

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Australia								
Blue grouper *Achoerodus viridis*	Aug-Sep 1990	Sydney	M/L	<5.6/310	0.80/8.1	0.70/1.5	1.5/23	Kannan *et al.*, 1995
Rubberlip morwong, *Nemadactylus douglasii*	Aug-Sep 1990	Sydney	M/L	13/54	0.80/9.0	6.2/2.0	20.2.0	Kannan *et al.*, 1995
Shovelnose ray, *Aptchotrema rostrata*	Aug-Sep 1990	Sydney	M/L	42/180	2.2/1.2	3.2/23	47/1.5	Kannan *et al.*, 1995
Long-spined snapper, *Argyrops spinifer*	Aug-Sep 1990	Perth	M	5.8	0.68	0.35	6.8	Kannan *et al.*, 1995
Sea mullet, *Mugil cephalus*	Aug-Sep 1990	Perth	M/L	<5.6/33	0.68/1.5	0.80/2.1	1.5/37	Kannan *et al.*, 1995
Spinytailed leatherjacket, *Bigener brownii*	Aug-Sep 1990	Perth	M/L	14/<14	1.8/1.3	3.4/16	19/17	Kannan *et al.*, 1995
Australian herring, *Arripis georgianus*	Aug-Sep 1990	Perth	M	23	2.4	1.9	27	Kannan *et al.*, 1995
Striped seaperch, *Lujanus vitta*	Aug-Sep 1990	Perth	M/L	23/52	1.1/1.5	0.73/2.1	25/56	Kannan *et al.*, 1995
Black bream, *Acanthopagrus bulcheri*	Aug-Sep 1990	Perth	M/L	24/<14	1.4/<0.9	0.21/1.5	26/1.5	Kannan *et al.*, 1995
Blue mussel, *Mytilus edulis*	1991	Perth	S	-	-	<1-330	-	Burt and Ebell, 1995

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Rainbow trout, *Oncorhynchus mykiss*	Aug-Sep 1990	Tasmania	M/L	20/<14	0.84/<0.9	0.49/1.9	21/1.9	Kannan *et al.*, 1995
Atlantic salmon, *Salmo salar*	Aug-Sep 1990	Tasmania	M/L	8.8/21	1.1/9.7	6.3/19	16/50	Kannan *et al.*, 1995
Sea mullet, *M. cephalus*	Feb-Mar 1992	Brisbane	M/L	9.2/260	1.4/12	1.5/6.3	12/280	Kannan *et al.*, 1995
Silver bream, *Acanthopagrus australis*	Feb-Mar 1992	Brisbane	M/L	10/<14	1.3/<0.9	0.39/1.2	12/1.2	Kannan *et al.*, 1995
Mud flathead, *Platycephalus fuscus*	Feb-Mar 1992	Brisbane	M/L	24/250	3.1/3.4	2.0/6.22	29/260	Kannan *et al.*, 1995
Silver travelly, *Caranx sexfasciatus*	Feb-Mar 1992	Townsvill	M/L	26/470	2.7/24	11/72	40/570	Kannan *et al.*, 1995
Spripey, *Lujanus carponotatus*	Feb-Mar 1992	Townsvill	M	<5.6	<0.36	<0.13	ND	Kannan *et al.*, 1995
Black pomfret, *Apolectus niger*	Feb-Mar 1992	Townsvill	M	10	1.7	13	25	Kannan *et al.*, 1995
Squid, *Loligo chinensis*	Feb-Mar 1992	Townsvill	M	<5.6	1.4	0.54	1.9	Kannan *et al.*, 1995
Sea bass, *Dicentrarcus labrax*	Feb-Mar 1992	Atherton	M/L	23/88	2.2/7.4	2.3/22	28/120	Kannan *et al.*, 1995
Bangladesh								
Flounder, *Wallaga attu*	Jan 1994	Dhaka	M	85	5.2	1.7	92	Kannan *et al.*, 1995
Catla, *Catla catla*	Jan 1994	Dhaka	M	170	15	1.5	190	Kannan *et al.*, 1995

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Hilsa, *Hilsha ilisha*	Jan 1994	Dhaka	M	9.9	1.7	3.0	15	Kannan *et al.*, 1995
Aor, *Mystus aor*	Jan 1994	Dhaka	M	<5.6	<0.36	0.69	0.69	Kannan *et al.*, 1995
Canada								
Blue mussel, *M. edulis*	1990	British Columbua	S	8.2-49	6.8-80	52-314	-	Stewart and Thompson, 1994
Denmark								
Soft-shell clam, *Mya areanaria*	1989	Fyn	S	-	-	250-1470	-	Kure and Depledge, 1994
Hong Kong								
Green mussel, *Perna viridis*	1989	Hong Kong	S	-	-	64-115	-	Chiu *et al.*, 1991
India								
Scombrid, *Scomberoides* sp.	Dec1989	Delhi	M	85	5.2	1.7	92	Kannan *et al.*, 1995
Catfish, *Clarius* sp.	Dec1989	Delhi	M	10	0.65	0.60	0.69	Kannan *et al.*, 1995
Jawfish, *Otolithus* sp.	Dec1989	Delhi	M	7.6	<0.36	1.6	9.2	Kannan *et al.*, 1995
Sciaenid fish, *Protonibea diacanthus*	Dec1989	Delhi	M	<5.6	<0.36	<0.13	ND	Kannan *et al.*, 1995

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Indian mackerel, *Rastrelliger. Kanagurta*	Dec1989	Delhi	M	<5.6	0.49	1.2	1.7	Kannan *et al.*, 1995
Prawns (pooled samples)[a]	Dec1989	Porto novo	M	22	0.41	0.13	23	Kannan *et al.*, 1995
Sea mullet, *M. caphlus*	Dec1989	Porto novo	M	18	<0.36	<0.13	18	Kannan *et al.*, 1995
Pearl spot, *Etroplus suratensis*	Dec1989	Porto novo	M	<5.6	<0.36	<0.13	ND	Kannan *et al.*, 1995
Catla, *C. catla*	Dec1989	Bombay	M	7.0	<0.36	<0.13	7.0	Kannan *et al.*, 1995
Silver pomfret, *Pampus argenteus*	Dec1989	Bombay	M	78	<0.36	1.2	79	Kannan *et al.*, 1995
Indian mackerel, *R. kanagurta*	Dec1989	Bombay	M	55	<0.36	1.2	56	Kannan *et al.*, 1995
Black bream, *Acanthopagrus* sp.	Dec1989	Calcutta	M	33	<0.36	<0.13	33	Kannan *et al.*, 1995
Threadfins, *Eleutheronema tetradactylum*	Dec1989	Calcutta	M	46	0.36	0.44	47	Kannan *et al.*, 1995
Perch, *Lates calcarifer*	Dec1989	Calcutta	M	40	<0.36	0.23	40	Kannan *et al.*, 1995
Catla, *C. catla*	Dec1989	Calcutta	M	41	<0.36	0.15	56	Kannan *et al.*, 1995

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Indonesia								
Big-eye scad, *Selar crumenophthalmus*	Nov 1991	Bogor	M	10	4.8	3.7	19	Kannan *et al.*, 1995
Deep-bodied crucian, *Carassius cuvier*	Nov 1991	Bogor	M	<5.6	0.41	<0.13	0.41	Kannan *et al.*, 1995
Japan								
Caprellid, *Caprella danilevskii*	Sep 1994	Otsuchi Bay	W	11	9.2	59	79	Takahashi *et al.*, 1999
Caprellid, *C. subinermis*	Sep 1994	Otsuchi Bay	W	17	15	57	89	Takahashi *et al.*, 1999
Caprellid, *C. equilibra*	May 1995	Otsuchi Bay	W	28	13	71	110	Takahashi *et al.*, 1999
Caprellid, *C. mutica*	May 1995	Otsuchi Bay	W	19	13	94	130	Takahashi *et al.*, 1999
Caprellid, *C. panantis* S-type	May 1995	Otsuchi Bay	W	24	17	140	180	Takahashi *et al.*, 1999
Caprellid, *C. panantis* R-type (male)	Aug 1995	Otsuchi Bay	W	11	8.7	58	78	Takahashi *et al.*, 1999
Caprellid, *C. panantis* R-type (female)	Aug 1995	Otsuchi Bay	W	17	7.9	73	98	Takahashi *et al.*, 1999
Gammarid, *Jassa* sp.	May, 1995	Otsuchi Bay	W	25	9.5	12	47	Takahashi *et al.*, 1999

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Mussel, *Mytilus galloprovincialis*	Sep 1994	Otsuchi Bay	S	9.4	34	45	88	Takahashi *et al.*, 1999
Blue mussel, *M. edulis*	1989	Tokyo Bay	S	20-120	40-540	20-240	-	Higashiyama *et al.*, 1991
Ascidian, *Halocynthia roretzi*	Sep 1994	Otsuchi Bay	W	<9.0	13	42	55	Takahashi *et al.*, 1999
Sea urchin, *Strongylocentrotus intermedius*	Sep 1994	Otsuchi Bay	S	<9.0	5.4-11	17-41	22-52	Takahashi *et al.*, 1999
Conger eel1, *Conger myriaster*	Sep 1994	Otsuchi Bay	M/L	<9.0-21/66	5.3-13/60	9.2-13/28	15-47/150	Takahashi *et al.*, 1999
Conger eel2, *C. myriaster*	Aug 1995	Otsuchi Bay	W	<9.0	8.6	14	23	Takahashi *et al.*, 1999
Greenling, *Hexagrammos otakii*	Sep 1994	Otsuchi Bay	M	12	2.1	4.2	18	Takahashi *et al.*, 1999
Morid cod1, *Physiculus maximowiczi*	Sep 1994	Otsuchi Bay	M/L/G	25/<9.0/12	5.1/11/4.6	4.2/8.5/9.9	34/20/27	Takahashi *et al.*, 1999
Morid cod2, *P. maximowiczi*	Aug 1995	Otsuchi Bay	W	<9.0	1.8	5.1	6.9	Takahashi *et al.*, 1999
Gunnel, *Pholis neblosa*	May 1995	Otsuchi Bay	W	<9.0	6.1	31	37	Takahashi *et al.*, 1999
Gunnel, *P. crassispina*	May 1995	Otsuchi Bay	W	11-15	8.8-32	61-210	81-260	Takahashi *et al.*, 1999

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Sculpin, *Pseudoblennius cottoides*	May 1995	Otsuchi Bay	W	<9.0	7.3	79	86	Takahashi *et al.*, 1999
Dall's popoise, *Phocoenoides dalli*	Feb 1995	Off Sanriku	L	97 (50-120)	430 (180-600)	230 (110-310)	760 (340-1000)	Takahashi *et al.*, 1999
Malaysia								
Green mussel, *P. viridis*	1992	Penmadu Malaysia	S			[illegible]		Tong *et al.*, 1996
Mediteranean								
Mussel, *M. galloprovincialis*	1989	Western coastal encloures	S	-	12-2450	220-2600	-	Tolosa *et al.*, 1992[c]
Mexico								
Oyster, *Crassostrea virginica*	1989	Gulf of Mexico	S	<5-145	<5-380	<5-1450	-	Gacia-Romeo *et al.*, 1993[c,d]
Oyster, *C. virginica*	1990	Gulf of Mexico	S	<5-25	<5-160	<5-7760	-	Gacia-Romeo *et al.*, 1993[c,d]
Oyster, *C. virginica*	1991	Gulf of Mexico	S	<5-42	<5-200	<5-1160	-	Gacia-Romeo *et al.*, 1993[c,d]
Papua New Guinea								
Sea mullet, *M. cephalus*	Aug-Sep 1990	Port Moresby	M	8.0	0.98	<0.13	9.0	Kannan *et al.*, 1995
Mud crab, *Scylla serrata*	Aug-Sep 1990	Port Moresby	M	<5.6	<0.36	<0.13	ND	Kannan *et al.*, 1995

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Tilapia, *Tilapia nilotica*	Aug-Sep 1990	Port Moresby	M/L	<5.6/33	<0.36/3.1	0.13/1.6	0.13/38	Kannan *et al.*, 1995
Oyster, *Ostrea* sp.	Aug-Sep 1990	Koki market	M	6.8	<0.36	0.15	7.0	Kannan *et al.*, 1995
Poland								
Flounder, *Platychthis flesus*	Jul 1990	Gdańsk Bay, southern Baltic Sea	M	-	-	-	390-632	Kannan and Falandysz, 1997
Erring, *Clupea harengus*	May 1990	Gdańsk Bay, southern Baltic Sea	M	-	-	-	40	Kannan and Falandysz, 1997
Eel, *Anguilla anguilla*	Aug 1990	Gdańsk Bay, southern Baltic Sea	M	-	-	-	188	Kannan and Falandysz, 1997
Sea trout, *Salmo trutta*	Apr 1990	Gdańsk Bay, southern Baltic Sea	M	-	-	-	51 (45-57)	Kannan and Falandysz, 1997
Turbot, *Psetta maxima*	Jul 1990	Gdańsk Bay, southern Baltic Sea	M	-	-	-	39	Kannan and Falandysz, 1997
Cod, *Gadus morhua*	Mar 1990	Gdańsk Bay, southern Baltic Sea	M	-	-	-	19 (14-24)	Kannan and Falandysz, 1997
Eelpout, *Zoarces vivparus*	May 1990	Gdańsk Bay, southern Baltic Sea	M	-	-	-	130	Kannan and Falandysz, 1997

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Pikeperch, *Stizostedion luciperca*	Aug 1990	Gdańsk Bay, southern Baltic Sea	M	-	-	-	455	Kannan and Falandysz, 1997
Mackerel, *Scomber scombus*	Jun 1990	Gdańsk Bay, southern Baltic Sea	M	-	-	-	27 (23-30)	Kannan and Falandysz, 1997
Red-throated diver [c] (male), *Gavia stellata*	Winter	Gdańsk Bay	L	-	-	-	610	Kannan and Falandysz, 1997
Razorbil [c] (male), *Alca torda*	Winter	Gdańsk Bay	L	-	-	-	330 (260-380)	Kannan and Falandysz, 1997
Great crest grabe [c] (male), *Podiceps cristatus*	Spring	Gdańsk Bay	L	-	-	-	540	Kannan and Falandysz, 1997
Black cormorant [c] (male), *Phalacrocorax carbo*	Winter	Gdańsk Bay	L	-	-	-	870	Kannan and Falandysz, 1997
Long-tailed duck [c] (female), *Clangula hyemalis*	Winter	Gdańsk Bay	L	-	-	-	4600	Kannan and Falandysz, 1997
Long-tailed duck [c] (male), *Clangula hyemalis*	Winter	Gdańsk Bay	L	-	-	-	280	Kannan and Falandysz, 1997
White taailed eagle [c] (female), *Haliaetus albicilla*	Winter	Gorzow Procince	L	-	-	-	35	Kannan and Falandysz, 1997
Guillemo [c] (male), *Uria aalge*	Winter	Gdańsk Bay	L	-	-	-	500 (430-590)	Kannan and Falandysz, 1997

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Portugal								
Mussel, *M.galloprovinciallis*	-	West coast	S	3.2-196	ND-82	ND-114	-	Quevauviller *et al.*, 1989
Solomon islands								
Greenspoted kingfish, *Caranx papuensis*	Aug-Sep 1990	Honiara	M	<5.6	<0.36	0.2	0.2	Kannan *et al.*, 1995
Indian mackerel, *R. kanagurta*	Aug-Sep 1990	Honiara	M/L	<5.6/77	0.38/5.3	1.0/6.5	1.4/89	Kannan *et al.*, 1995
Paddletail snapper, *Lutjanus gibbus*	Aug-Sep 1990	Honiara	M	<5.6	0.40	0.39	0.79	Kannan *et al.*, 1995
Taiwan								
Tilapia, *T. nilotica*	Aug 1990	Taipei	M	<5.6	0.36	0.13	0.49	Kannan *et al.*, 1995
Milkfish	Aug 1990	Taipei	M	<5.6	0.75	0.21	0.96	Kannan *et al.*, 1995
Seabream, *Gymnocranius elongatus*	Aug 1990	Taipei	M	11	2.1	5.2	18	Kannan *et al.*, 1995
Bivalve (pooled 40 samples)	Aug 1990	Taipei	M	13	1.5	1.7	16	Kannan *et al.*, 1995
Thailand								
Silver pomfret, *P. argenteus*	Jan 1994	Bangkok	M	<5.6	1.6	1.3	2.9	Kannan *et al.*, 1995

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Indian mackerel, *R. Kanagurta*	Jan 1994	Bangkok	M	<5.6	2.3	2.1	4.4	Kannan *et al.*, 1995
Giant seaperch, *L. calcarifer*	Jan 1994	Bangkok	M	<5.6	2.6	13	16	Kannan *et al.*, 1995
Green mussl, *P. viridis*	Sep 1994	Trat River, Trat	S	<3	1	8	12	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Trat River, Trat	S	<3	2	11	16	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Kung Kra Baen, Chanthaburi	S	42	80	680	802	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Sep 1994	Ban Pae, Rayong	S	38	10	25	73	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Jan 1994	Sichang Island, Chonburi	S	45	66	200	311	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Ang Sila, Chonburi	S	3	5	24	32	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Sep 1994	Chao Phraya river, Samut Prakan	S	7	10	56	73	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Chao Phraya river, Samut Prakan	S	8	9	48	65	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Sep 1994	Tachin River, Samut sakhon	S	<3	3	25	31	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Tachin River, Samut sakhon	S	<3	2	9	14	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Sep 1994	Ban Laem, Phetchaburi	S	5	7	7	19	Kan-atireklap *et al.*, 1997b

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Green mussl, *P. viridis*	Mar 1995	Ban Laem, Phetchaburi	S	<3	4	23	30	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Sep 1994	Muang, Phachuap Khiri Khan	S	9	20	130	159	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Muang, Phachuap Khiri Khan	S	7	16	210	233	Kan-atireklap *et al.*, 1997b
Green mussl, *P viridis*	Mar 1995	Paknam, Chumphon	S	3	3	11	17	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Pak Nam Kra Dae Surat Thani	S	4	8	49	61	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Songkhla Lake, Songkhla	S	3	5	27	35	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Muang, Pattani	S	5	6	41	52	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Yong Star, Trang	S	<3	8	89	100	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Bang Toey, Pang Nga	S	<3	1	3	7	Kan-atireklap *et al.*, 1997b
Green mussl, *P. viridis*	Mar 1995	Ao Makham, Phuket	S	<3	4	28	35	Kan-atireklap *et al.*, 1997b
United Kngdom								
Soft-shell clam, *Mya areaaria*	1987	Poole harbour	S	-	1780-9950	-	-	Langston *et al.*, 1987[c,d]

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
United States								
Blue mussel, *M. edulis*	1986-1987	Pacific coast	S	-	-	<5-1080	-	Short and Sharp, 1989
Blue mussel, *M. edulis*	1988-1990	East coast	S	ND-140	10-580	10-1200		Uhler *et al.*, 1993
Blue mussel, *M. edulis*	1988-1990	West coast	S	ND-300	10-740	10-1380	-	Uhler *et al.*, 1993
Blue mussel, *M. edulis*	1988-1900	East coastal	S	ND-330	10-1570	10-4030	-	Uhler *et al.*, 1993
Oyster, *C. virginica*	-	Chesapeak bay	S	-	-	<10-5600	-	Espourteille *et al.*, 1993
Vietnam								
Silver carp, *Hypophthalmus molitrix*	Jan 1990	Hanoi	M	<5.6	<0.36	<0.13	ND	Kannan *et al.*, 1995
Carp, *Ctenopharyngodon* sp.	Jan 1990	Hanoi	M	<5.6	<0.36	<0.13	ND	Kannan *et al.*, 1995
Bream, *Evynnis japonica*	Jan 1990	Phu Da	M	<5.6	0.73	0.2	0.93	Kannan *et al.*, 1995
Sea mullet, *M. cephalus*	Jan 1990	Ho chi Minh	M	<5.6	<0.36	<0.13	ND	Kannan *et al.*, 1995
Perch, *L. calcarifer*	Jan 1990	Ho chi Minh	M	<5.6	<0.36	0.24	0.24	Kannan *et al.*, 1995
Tilapia, *Tilapia nilotica*	Jan 1990	Ho chi Minh	M	<5.6	<0.36	0.23	0.23	Kannan *et al.*, 1995

Table 2.4 cont.

Country/Species	Collected month and year	Locations	Tissue/organ[a]	MBT	DBT	TBT	ΣBT[b]	References
Prawn, *Penaeus monodon*	Jan 1990	Ho chi Minh	M	<5.6	<0.36	0.15	0.15	Kannan *et al.*, 1995
Shellfish, *Scapharca subcrenata*	Jan 1990	Ho chi Minh	M	<5.6	<0.36	0.90	1.7	Kannan *et al.*, 1995
Striped snakeheadfish, *Ophicephalus striatus*	Jan 1990	Cu Chi	M	<5.6	0.78	<0.13	ND	Kannan *et al.*, 1995

[a]Tissue/organs were showed as: M (muscle), L (liver), G (gill), S (whole soft tissue), and W (whole body)

[b]$\Sigma BT = MBT + DBT + TBT$

[c]Dy wt basis

[d]Express in Sn

[e]Fish-eating birds

Data presented as mean and/or range; ND—not detected

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2.8 Contamination of TBT in biota

The presence of alkyl groups on the tin atom of TBT leads to an increased in its lipophilic properties, and thereby in an increased capacity to become bioaccumulated in living organisms. Theoretically, the octanol/water partition coefficient (K_{ow}) provides the possibility of calculating the bioconcentration factor (BCF). TBT contamination in some aquatic organisms is presented as BCF in Table 2.5 below.

Table 2.5 Boiconcentration factors (BCFs) for TBT in aquatic organisms

Species	BCF	References
Microorganisms		
Estuarine bacteria	356-1039	Alzieu, 1996
Pseudomonas 224	438-487	Alzieu, 1996
Phytoplantons		
Isochrysis galbana	5500	Alzieu, 1996
Ankistodesmus falcata	30000	Alzieu, 1996
Mollusks		
Pacific oyster, *Crassostrea gigas*	2000-6000/16000	Alzieu, 1996
	25000	Shim *et al.*, 1998
European flat oyster, *Ostreas edulis*	1000-1500	Alzieu, 1996
Blue mussel, *Mytilus edulis*	1500-7300	Laughlin and French, 1988
	7000-19000	Zuolian and Jensen, 1989
	10000	Suzuki *et al.*, 1998*
Mussel, *M. graynus*	11000	Suzuki *et al.*, 1998*
Mussel, *M galloprovincialis*	11000	Takahashi *et al.*, 1999
Dog-whelk, *Nucella lapillus*	29000	Bryan and Gibbs, 1991
Gmelin, *N. lima*	2200-2900	Stickle *et al.*, 1990
Rock shell, *Thais clavigera*	5000-10000	Horiguchi *et al.*, 1995
Stenoglossan prosobranch, *Hinia incrassata*	4680-122000	Oehlmann *et al.*, 1998
Soft-shelled clam, *Mya arenaria*	5700-220000	Kure and Depledge, 1994

Table 2.5 cont.

Species	BCF	References
Clam, *Ruditapes decussata*	12000	Morcillo and Porte, 2000
Crustaceans		
Gammarid, *Rhepoxynius abronius*	360[b]	Meador *et al.*, 1993
Gammarid, *Eohaustorius esturius*	6300[b]	Meador *et al.*, 1993
Gammarid, *Jassa* sp.	2700	Takahashi *et al.*, 1999
Caprellids	14000-33000[c]	Takahashi *et al.*, 1999
Mud crab, *Rhithropanopeus harissii*	500-4400	Alzieu, 1996
Fish		
Sheephed minnow, *Cyprinodon variegatus*	2600	Takahashi *et al.*, 1999
Rainbow trout, *Salmo gairdneri*	410	Martin *et al.*, 1989
Guppy, *Lebistes teticulatus*	240-460	Tsuda *et al.*, 1990
Minnow (larvae), *Phoximus phoxumus*	410-540	Fent, 1991
Mullet, *Mugil cephalus*	2300-3000	Yamada *et al.*, 1992
Filefish, *Ruarius ercodes*	3200-3600	Yamada *et al.*, 1992
Red sea bream, *Pagrus major*	9400-11000	Yamada *et al.*, 1992
Graying (larvae), *Thymallus thymallus*	2000	Fent and Looser, 1995
Morid cod, *Physiculus maximowiczi*	1200	Takahashi *et al.*, 1999
Conger ell, *Conger myriaster*	3300	Takahashi *et al.*, 1999
Gunnel, *Pholis crassispina*	19000-50000	Takahashi *et al.*, 1999
Atlantic salmon, *Salmo salar*	0.9 (kidney), 3.9 (liver)	Alzieu, 1996
Mullet, Liza aurata	3-19.6	Alzieu, 1996

[a]Data cited form different sources and in ascending years
[b]BCFs estimated with the assumption of 80% of moisture content in the tissue of organisms.
[c]Minimum BCF in *Caprellia subinermis* to maximum in *C. penantis* S-type

2.9 Effects on aquatic organisms

TBT toxicity was reported firstly in pacific oyster (*C. gigas*) in France, the significant toxic effects are compiled in this section by the major groups of aquatic organisms. It induces abnormal shell termed "chambering". Besides *C. gigas*, induced shell curl also occurs in other oyster species, such as the Portuguese oyster *C. angulata* (Phelps and Page, 1997).

2.9.1 Lethal toxicity

Due to the wide range of TBTs activities to aquatic species, TBT compounds can cause mortality in microorganisms only in order of nanogram per litre of concentrations and in the levels of microgram per litre reach the lethal concentration in aquatic vertebrate. The Acute toxicity of TBT compounds to freshwater organisms is summarized by Maguire (1996). In addition, lethal and sublethal effects of TBT compounds for marine organisms are summarized by Alzieu (1996).

Lethal toxicity of TBTs were conducted in some crustaceans: 96 h LC_{50}s of 13.0 and 33.6 $\mu g\ l^{-1}$ have been found for mud crab zoea, *Rhithropanopeus harrissii*, respectively collected in California and Florida (Laughlin and French, 1989). In *P. japonicus*, 96 h LC_{50}s were 19.4 and 93.6 $\mu g\ l^{-1}$ in PL5 and PL15, respectively (Lignot *et al.*, 1998). As regards survival of larvae, a concentration of 9.5 $\mu g\ l^{-1}$ decreases survival rate of *Hemigrapsus nudus* by 50% after 6.2 days of exposure (Laughlin and French, 1980). In the case of juvenile and adult instars the 96 h LC_{50}s were: over 31 μg TBT l^{-1} in the grass shrimp *Palaemonetes* sp. (Buchong *et al.*, 1988) and 370 $\mu g\ l^{-1}$ in *P. japonicus* (Lignot *et al.*, 1998).

2.9.2 Sublethal toxicity

Microphytes

Regarding to environmental concerns, TBT compounds contamination is considered in primary productivity deterioration. In addition, it can consequently affect the higher trophic levels. TBT inhibited photosynthesis of periphyton with the estimated non-observable effective concentration (NOEC) value of 0.5 nM. By treating with TBTCl or TBTO, the toxic effect is exerted by the same dissociation product, most likely the TBT cation (Blanck and Dahl, 1996).

Mollusks

Sublethal concentrations of TBT potentially induced the well-known abnormality in sensitive female gastropods, which exhibit male characteristic. The term "imposex" is used to recognize this condition, and call the affected female gastropods as "imposers". At least 72 species of gastropod mollusks are known to be affected by TBT compounds at many locations worldwide (Evans *et al.*, 1995). For example, *Ilyanassa* obsoleta (Obersdorster, Rittschof and Mcclellan-Green, 1998), *Oceneebra erinacea* (Gibbs, 1996), *Nucella lapillus* (Evans, Kerrigan and Palmer, 2000; Son and Hughes, 2000), *N. emarginta* (Tester, Ellis and Thompson, 1996), *Thais clavigera* (Lie *et al.*, 1997; Tan, 1997), *T. bitubercularis* (Bech, 1999). A related condition referred to as "intersex" has been reported in littorinid mesogastropods and these to become unable to lay eggs (Buaer *et al.*, 1997; Horiguchi *et al.*, 1997; Matthiessen and Gibbs, 1998). Intersex can be induced only in juvenile and sexually immature females and its intensity depends on the ontogenic stage of development during TBT exposure (Bauber *et al.*, 1997). This effect has been utilized for monitoring and evaluation TBT contamination.

Physiological and biochemical phenomena leading to imposex are still not well understood. Nevertheless, there are evidences the TBT exposure tends to increase the testosterone contents in female mollusks (Morcillo and Porte, 2000; Obersdorster, Rittschof and Mcclellan-Green, 1998), while progesterone and 17E oestradiol levels remain constant. Since testosterone content alone causes penis growth in the females, it is thought that imposex could be attributed to its accumulation originating from inhibition of cytochrome P450-dependent aromatase (Mattiessen and Gibbs, 1998). The conversion of testosterone into 17E oestradiol would then inhibited by TBT (Azieu, 2000).

Crustaceans

Tributyltin causes alterations in testosterone metabolism in daphnids *Daphnia magna* that would result in an increase in the production of oxide-reduced derivatives, these products are preferentially retained in the tissues of daphnids and are variously androgenic in vertebrates. The increased production of oxido-reduced derivatives of testosterone may be mechanically responsible for the masculinizing effects of TBT in some species (LeBlanc and McLachan, 2000). In case of peneid shrimp, *Penaeus japonicus*, TBTO decreased the osmoregulatory

capacity of exposing animals by histopathological effects on gills and epipodites. Tolerance to TBTO was increase with developmental stages (Lignot *et al*, 1998). In blue crab, *Callinectes sapidus*, respiration rates were significantly decreased after TBT exposure, microsomal P450 protein in hepatopancreas was interfered after fed with TBT-treated fish for 16 days (Oberdorster, Rittschof and McClellon-Green, 1997). At a concentration of 1 ng TBT l^{-1}, developmental rate of copepod larvae, *Acartia tonsa*, was inhibited (Kusk and Petersen, 1997).

Horseshoe crab

Horseshoe crab *Limulus polyphemus* larvae exhibited relatively very high tolerance to TBT compared to other aquatic organisms with the LC_{50}s of >1,000, 752 and 594 μg l^{-1} for 24-, 48- and 72 h exposure, respectively. Acute exposure to TBT significantly the time required by larvae to molt into the first-tail stage. For embryos, the earlier developmental stage was about 30-40 fold more susceptible to TBT than larvae. The LC_{50} for horseshoe crab embryos exposed to TBT were 44, 20 and 14 μg l^{-1}, respectively for 24-, 48- and 72 h acute exposure. The ability of horseshoe crab embryos and larvae to survive in the presence of organotin pollution suggests the possibility of bioaccumulation and movement into the estuarine food chain via seabirds, gulls, and fish (Botton, Hodge and Gonzales, 1998).

Echinoderm

Nanomolar concentrations of TBT (5×10^{-10} to 5×10^{-9}M) could directly affected sea urchin *Paracentrolus lividus* egg development after fertilization. Preincubation enhanced TBT toxicity to first cleavage DNA and protein synthesis but not intracellular calcium sequestration. At the range of concentrations, TBT caused arm length reduction and diameter increase of the rudiment in exposing larvae (Girard *et al.*, 2000).

Hemichordates

In the colonial ascidian *Botryllus schlosseri*, TBT acts as immunotoxic resulting in cytoskeletal alteration at a sublethal concentration of 10 ?M. The main mechanism of structural damage to cytoskeletal components is hypothesized that alteration of Ca^{2+} homeostasis by means of direct interaction with endogenous calmulodin (CaM), with induces a conformational change

preventing the regulative activity of CaM on Ca^{2+}-ATPase. Consequently, an excess of cytosolic Ca^{2+} accumulation that, together with the inhibition of CaM-dependent kinases and Ca^{2+} regulates proteins produces extensive cytotskeletal disorganization (Cima and Ballarin, 2000). In addition, TBT also profoundly affected natural immune reaction in tunicate such as phagocytosis, cellular toxicity, and hematopoietic cell proliferation. The effects were exerted by altering the relative frequencies of circulatory hemocytes (Paftos and Hutchinson, 1997).

Fish

TBT could lessen hatching success of embryos, and also caused malformation in medaka or red killifish *Oryzias lapites* hatchlings consisted of tail bent at the tip, curled, and/or shortened which corresponded with statistically significant reduction in number of somites. Moreover, developmental rate was slowed by TBT in a concentration-related manner but not depend on age of exposing embryo (Bentivegna and Piatkowski, 1998).

2.9.3 Mechanism of action for TBTO toxicity in shrimps

No other evidence has been established the pathological causes of mortality by TBTO in shrimp to date except the study on juvenile zebra shrimps *Penaeus japonicus* (Lignot *et al.*, 1998). One of the physical functions of the shrimp targeted by TBTO is osmoregulation. Treated shrimps were induced to loss the hypo- and hyper osmoregulatory capacity at both the lethal and sublethal concentrations, which might due to the histopathological injuries, appeared in gill lamellae and epipodites. Multiple necrosis, haemocytic congestion in the efferent and afferent vessels and severe nephrocyte (podocyte) hyperplasia were observed in gill lamellae (Fig. 2.6b, c,d) versus in control gill filament (Fig. 2.6a). Multiple necrosis and vacuolization were also observed in epithelial monolayers on most epipodites, which treated with the sublethal concentration of 200 $\mu g\ l^{-1}$ (Fig. 2.6f) compared to control epipodites (Fig. 2.6e). At lethal concentrations of 300 $\mu g\ l^{-1}$, epithellial cell were severely damaged (Fig. 2.6g). At the highest lethal concentration of 400 $\mu g\ l^{-1}$, the internal structures of the epipodites were also severely damaged. Between the two layers of epithelial cells, the interconnecting lacunae interspersed between the pilar cells and through which the hemolymph passes were reduced and/or replaced by proliferating tissues. Epithelial cells were peeling and oedema was observed (Fig. 2.6h).

concentrations of 300 µg l^{-1}, epithellial cell were severely damaged (Fig. 2.6g). At the highest lethal concentration of 400 µg l^{-1}, the internal structures of the epipodites were also severely damaged. Between the two layers of epithelial cells, the interconnecting lacunae interspersed between the pilar cells and through which the hemolymph passes were reduced and/or replaced by proliferating tissues. Epithelial cells were peeling and oedema was observed (Fig. 2.6h).

The effects of TBTO on gill structures of the exposed shrimps might be one of the main factors responsible for the loss of hypo- and hyper OC. The gills and epipodites is probably, with the digestive tract, the main tissue through which TBTO enters the shrimps from the water and histological damages can explain the imbalance in the plasma ion concentrations.

The sever injuries observed in gill lamellae and epipodites can alter the surface permeability to water and/or to ions, and therefor can be the factor responsible for the loss of the osmoregulatory capacity.

Nevertheless, there is necessary to further study the exact organ(s) targeted by TBTO that lead to osmoregulation disruption.

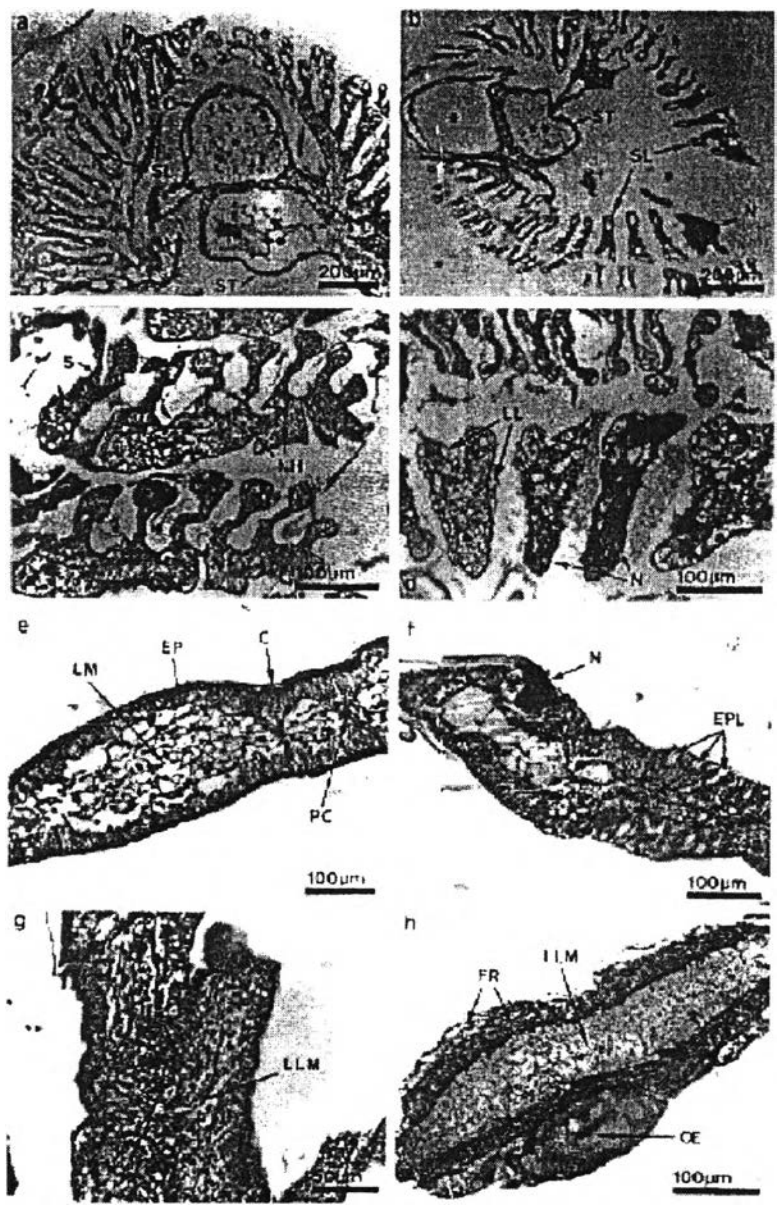

Fig. 2.6 Histopathological effects of TBTO on gills and epipodites of *Peneaus japonicus*. (a):gill filament (cross-section); (b): cross-section of a juvenile gill after 96 h exposure to 200 µg l^{-1} of TBTO; (c): gill lamellae of a shrimp exposed to 300 µg l^{-1} of TBTO for 96 h; (d): gill lamellae of a shrimp exposed to 400 µg l^{-1} of TBTO for 96 h; (e): longitudinal section of a control epipodite; (f): epipodite of a shrimp exposed to 200 µg l^{-1} TBTO for 96 h; (g): epipodite of a shrimp exposed to 300 µg l^{-1} of TBTO for 96 h; (h): epipodite of a shrimp exposed to 400 µg l^{-1} TBTO for 96 h. C, cuticle; EP, epithelial cells; EPL, epithelial cell lacunae; FR, fragment of cuticle; LL, lateral lacunae; LLM, loaded lacunae meskwork; LM, lacunae meshwork; N, necrosis; NH, nephrocite hyperplasia; OE, oedema; PC, pilar cell; S, septum; SL, secondary lamellae; ST, stem; *cross section of afferent vessel; **cross-section of efferent of stem. From Lignot *et al* (1998).

2.10 Test animal

The giant freshwater prawn *Macrobrachium rosenbergii* (Fig. 2.7), a familiar edible aquatic species, is widely distributed in the most of tropical and subtropical areas of Indo-Pacific region, including East Pakistan, India, Ceylon, Burma, Thailand, Malaysia, Indonesia, Philippines, Cambodia and Vietnam. In addition, it occurs extending to Northern Australia as well. The temporal existence is the whole year round and is present in both fresh and brackish waters.

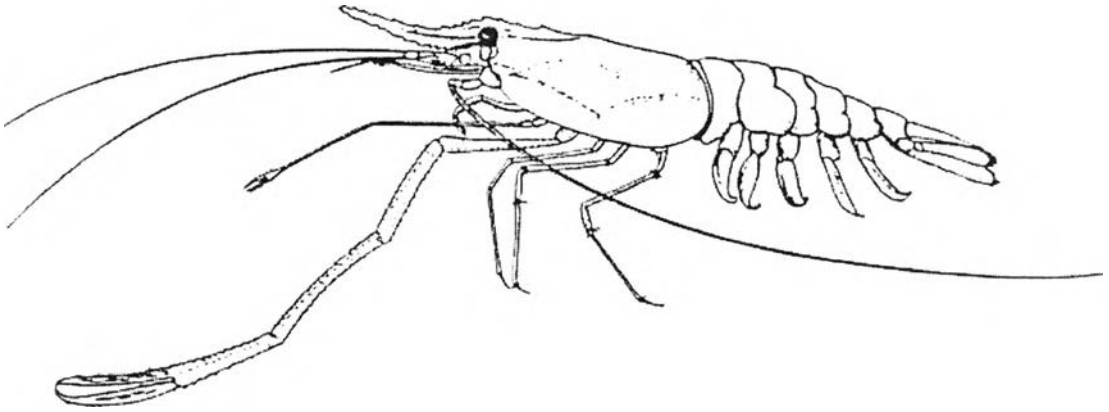

Fig. 2.7 A mature male giant freshwater prawn *Macrobrachium rosenbergii* de Man. Modified from Lee and Wickins (1992).

In Thailand, the zoogeographical distribution currently prepared by Naiyanetr (1998) is presented here as the following provincial localities: Bangkok, Nonthaburi, Pathum thani, Pra Nakorn Si Ayutthaya, Lob Buri, Ang Tong, Chainat, Suphan Buri, Nakorn Sawan, Nakorn Pathom, Samut Sakorn, Samut Songkhram (Central Thailand); Chacheongsao, Rayong, Chantaburi, Trat, Prachin Buri (East Thailand); Petchaburi (West Thailand); Chumporn, Surat Thani, Nakorn Si Thammarat, Trang, Songkhla, Pattani, Yala, Narathiwat, Phattalung, Phuket, Phanggna (South Thailand).

It dwells in various sources of water both in natural inland systems, in North Thailand, it exists in Moey River, the tributary of Salawin River of Mianmar, at Mae Sort, Tak. In Central plain, it is found throughout the length of Chao Phraya River, Ta chin River, Mae Klong River, Bang Pakong River, Pran Buri River, and Nakorn Nayok River. In East Thailand, it distributes in Chantaburi River, Weru River, Rayong River, and Trat River. In South Thailand, it presents in Lang Suan River, Tapi River, Kraburi River, Trang River, Pattani River and Songkhla Lake for

estuarine habitat. In case of anthropogenic habitat, it can be found in rice paddies (Yont Musik, 1986).

M. rosenbergii is a member belong to the genus which characterized by Fritz Müller as "*Macrobrachium* Sp. Bate". Etymologically, the genus name is derived from two words of Greek, *makros*, which means long but often mistakenly used to mean large, and be latinized to macro. The other word, *brachium*, is originated from *brachion* which means arm. The name refer to the enormous size of the second pair of pereiopods in males of the type species (Holthuis, 1993). This genus occurs throughout the tropics and in several subtropical areas. Almost all species pass at least part of their life in freshwater, in several juveniles are found in brackish water. Many of the species (there are about 125 species known at present) are of good size and it is likely that most will be used as food wherever they occur (Holthuis, 1980).

The taxonomic classification systemized by Naiyanetr (1998) is arranged as the followings, as well as the names of designator and the years of nomination.

Phylum *Arthropoda*
Class *Crustacea*, Pennant, 1797
Order *Decapoda* Latreille, 1803
Suborder *Pleocyemata* Burkenroad, 1963
Infraorder *Caredea* Dana, 1852
Superfamily *Palaemonoidea* Rafinesque, 1815
Family *Palaemonidae* Rafinesque, 1815
Genus *Macrobrachium* Bate, 1868
Species *Macrobrachium rosenbergii* de Man, 1879

The scientific synonyms of the species is given by many taxonomists as *Palaemon carcinus rosenbergii* Ortman, 1891; *Palaemon whitei* Sharp, 1893; *Palaemon* (*Eupalaemon*) *rosenbergii* Nobili, 1899; *Palaemon spinipes* Schenkel, 1902; *Palaemon dacqueti* Sunier, 1925; *Cryphiops* (*Macrobrachium*) *rosenbergii* Johnson, 1966. In older literature the species is often, but incorrectly, indicated with the name *Palaemon carcinus*.

The common names preferably used by FAO are "giant river prawn", "bouquet géant", and "camarón gigante", respectively in English, French, and Spanish. Moreover, the prawn bears

a variety of names in different parts of its ranges, such of those are "giant freshwater shrimp" or "—prawn", which is used in USA. Another, employed in both Calcutta, India and Bangladesh, is "Golda chingri" or "Mocha chingri". In Bangladesh, there are another names given to this prawn, "Bharo chingri" (or "Bara chingri"), "Chooan chingri", "Mota chingri", and "Shala chingri". In Java, Indonesia, the names in use are "Udang satang" and "Udang duri". For Malaya, Borneo, Indonesia, the prawn is called "Udang galah". Philippinoes call this prawn, "Hipon". In Cambodia, the local name for the prawn is "Bangkang". In Northern Australia, the local name in Aborigines is "Cherabin". In Japan the vernacular name applied to this prawn is "Onitenagaebi". The name in use by Danish is "Felsengarnele". And "Risa ferskvatnsrœkja" is the local name in Israel.

This prawn is known by a variety of names in different parts of its Thailand range. The usually employed vernacular name is "Kung kam kram" (molar claw prawn), in allusion to the raising and moving long claws in its territorial claim behaviour in muture male. This name is probably derived from the mispronounced vocative, "Kung kam khram", (indigo claw prawn) which closely mirrors its prominent characteristic. The prawn is sometimes called "Kung kam tong" (kam tong, golden claw) in allusion to brassy yellow claws of some caught individuals. A name frequently used in fish market is "Kung mae nam" (river prawn), perhaps to distinguish it from culture stocks. Another name very often in use is "Kung nang" (nang, lady). "Kung Yai" (big prawn) is also in use. In addition, "Kung luang" (luang, royal) is the name rarely applied to this prawn.

Briefly, embryos consumes about 19 days for incubation at 26 to 28 °C, the bright orange eggs gradually become lighter in colour and turn grey with progressive development until deepen to slate grey, when the larvae inside are fully developed (Ling, 1969a). The development of the embryos over incubation period are summarized in Table 2.6. The figures of some embryo developmental stages are illustrated in Fig 2.8.

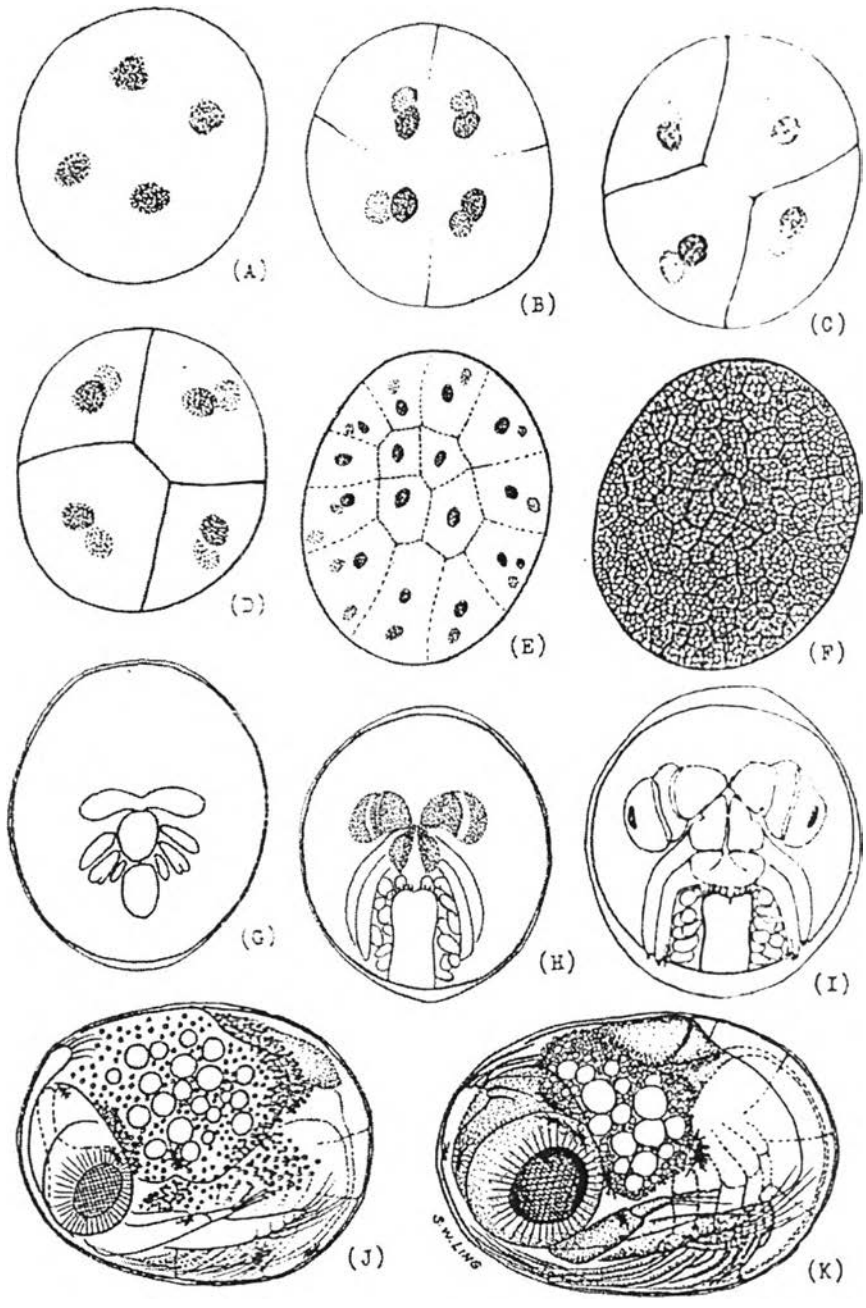

Fig. 2.8 Segmentation and embryonic development. Times refer to period since fertilization. (A) 7 h—completion of second nuclear division. (B) 8h 45 min—third nuclear division newly complete. (C) 8 h 55 min— third nuclear division complete, tips of the 4 cleavage furrows have met at 2 points from which the median furrow is developing. (D) 9 h—complete formation of 4 quadrants (blastomeres). (E) 14 h—32 nuclei. (F) 24 h—completion of segmentation. (G) 6 days—formation of caudal papilla. (H) 7 days—formation of optic vesicle (I) 9 days—eye pigment developed. (J) 14 days—larva fully formed. (K) 19 days—larva ready to hatch. From Ling (1969a).

Dissimilarly, penaeids hatch as nauplii and metamorphosis into zoea, mysis, and postlarva instars, while *M. rosenbergii* passes its nauplius stage within egg membrane and shows zoeal charateistics when hatching as free swimming larva (Waterman, 1960).

In case of larvae, the development spends averagely 31 days to reach the last stage (Uno and Kwon chin soo, 1969). The classification of developmental stages in *M. rosenbergii* was independently performed by Ling and Uno and Kwon chin soo in 1969. There are different in staging with the specific characteristics of larvae between the authors. Uno and Kwon chin soo reported eleven stages of development over larval instars (Fig 2.9), while Ling found only eight stages for larvae to metamorphosis into juveniles. Sixth, seventh and eighth stages of Uno and Kwon chin soo's are combined to seventh stage of Ling's. Ninth and both tenth and eleventh of Uno and Kwon chin soo's resemble to seventh and eighth stages described by Ling, respectively. Comparison of larval staging conducted by the authors are differentiated as Table 2.7

Table 2.6 Embryonic development of *Macrobrachium rosenbergii*

Time from extrusion	Status
3 hours	Stellate island of protoplasm containing the nucleus is clearly visible
4 hours	First nuclear division
6 hours	Second nuclear division
8 hours	Third nuclear division
14 hours	Fifth nuclear division (32 nuclei)
24 hours	Segmentation is completed
2 days	Gastulation
3 days	formation of prostomial lopes (nauplius embryo stage)
4 days (80 hours)	formation of buds of the nauplius appendages
5 days	formation of nauplius segment
7 days	formation of optic vesicle
9 days	formation of carapace rudiment
17 days	larva is fully developed
19-20 days	larva is ready to hatch

Source: summarized from Ling (1969a)

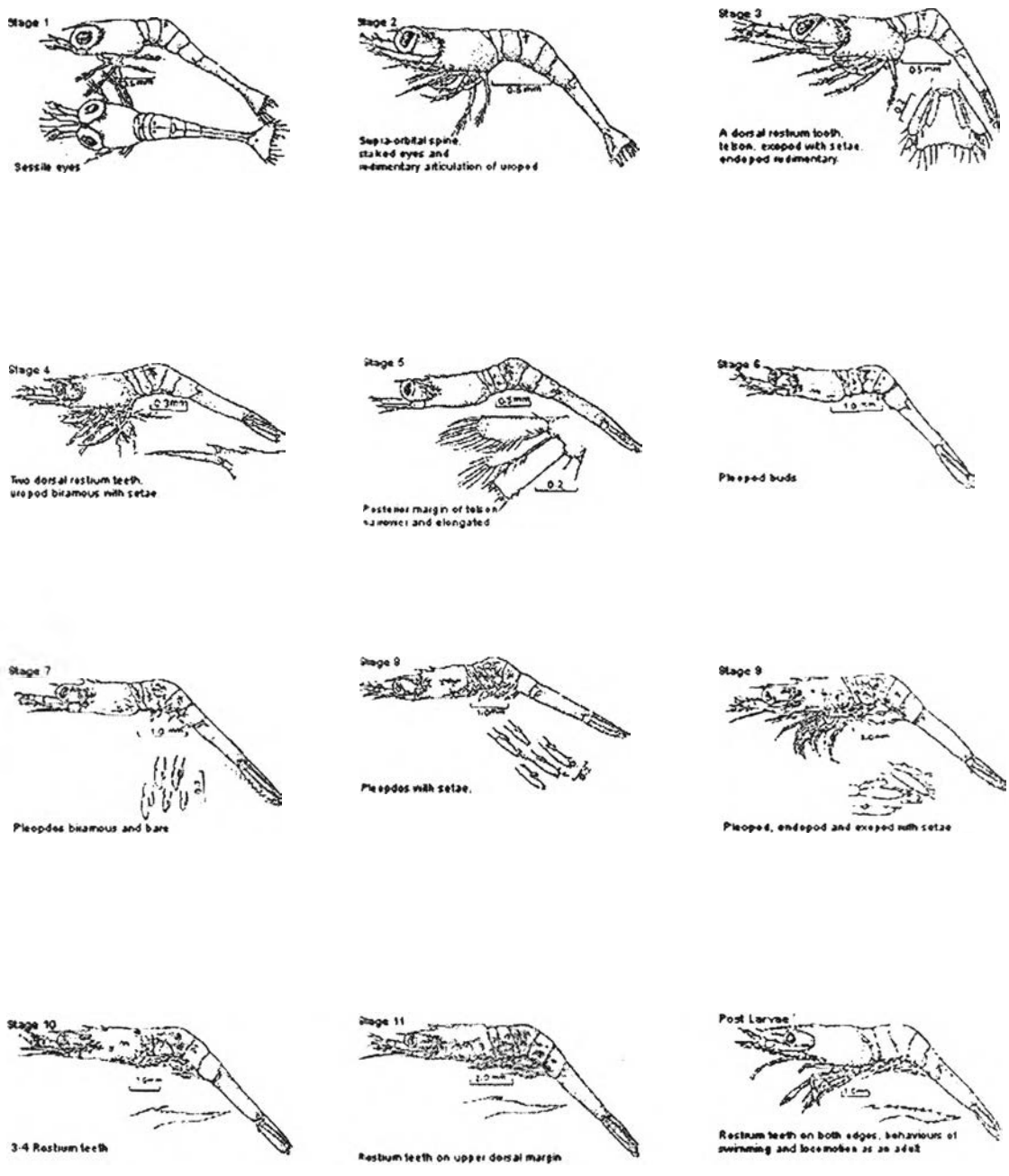

Fig. 2.9 Larval development of *Macrobrachium rosenbergii* de Man (modified from Uno and Kwon chin soo, 1969).

Table 2.7 Larval development of *Macrobrachium rosenbergii* by Ling (1969) versus Uno and Kwon chin soo (1969)

Ling (1969)				Uno and Kwon chin soo (1969)			
Stage	Age (days)	Body length (mm)	Keystone recognition		Body length (mm)	Age (days)	Stage
1	0 (1-2)	1.92 ± 0.02	Sessile eyes	Sessile eyes	2.0-2.2	1-2	1
2	2 (2-3)	1.99 ± 0.06	Supra-orbital spine, staled eyes and rudimental articulateion of uropod	Supra-orbital spine, staled eyes	2.3-2.4	3-5	2
3	4 (3-5)	2.14 ± 0.05	A dorsal rostrum tooth, sixth abdominal somite separated, uropod appeared	One epigastric spine behind base of rostrum, uropods presented, outer lopes two timeslonger than the inners	2.4-2.8	5-8	3
4	7 (5-9)	2.50 ± 0.08	Two dorsal rostrum teeth, uropod biramous with setae, chromatophores on second periopod merus	Distinct red and blue pigment on thoracic leg 2, two dorsal spines, endopods well developed	2.9-3.0	8-12	4
5	10 (9-12)	2.84 ± 0.07	Posterior margin of telson narrower and elongated, chromatophores prominent on mid-ventral abdomen	Telson rectangular, twice as long as board	3.2-3.3	11-17	5
6	14 (12-18)	3.75 ± 0.37	Buds of pleopod, telson more narrower, elongated terminally	Pleopod buds on abdominal segment 1 to 5, differences in development form specimen to specimen; 2nd, 3rd, and 4 th pairs usually more advance, larger bud biramous, telson much elongated	3.4-3.5	15-24	6
7	17 (15-20)	4.06 ± 0.15	Pleopods biramous, bare; outer attenular flagellum with four aesthetes on folded appendix				
8	20 (21-29)	4.68 ± 0.20	Pleopods with setae; incomplete chela				
9	24 (21-29)	6.07 ± 0.29	Pleopods, endopods, and exopods with setae	All five pairs of pleopods biramous, exopod and endopod both with plumuse setae	4.0-4.5	22-23	7
10	28 (25-34)	7.05 ± 0.52	Three or four dorsal rostrum teeth; middle dorso-lateral spines of telson disappeared	Rostrum with four to nine teeth on upper margin	5.0-5.8	30-45	8
11	31 (28-37)	7.73 ± 0.81	Rostrum teeth on upper dorsal margin				