Chapter 5

Conclusions and Recommendations

5.1 Conclusions

Present study indicates that TBTO is a lethal and non-teratogenic toxicant for *M. rosenbergii* ontogeny. TBTO considerably lessen the hatching success of *M. rosenbergii* embryos, between the range of 3.5-57 μg TBTO l^{-1} for 16 day of exposure the tolerance trends to increase with progressive development of embryos over the incubation period. Conversely, larvae rather displayed a consistent response to TBTO for first eight stages out of eleven instars of prejuvenile. By this manner, whichever stages can be selected to represent a sensitivity to a toxicant. It could say that teratogenesis is not the mode of action for TBTO in *M. rosenbergii.* Although the embryos can stand relatively high doses of TBTO compared to currently levels in some foreign areas, it does not ensure the recruitment status for this species under natural condition due to lack of knowledge in TBT contamination in Thai riverine system. Furthermore, higher concerns should be also paid on distribution pattern and contamination profile in estuaries where larvae spend certain part of their life there. More information and research should be collected and performed are proposed in the next section.

5.2 Recommendations

5.2.1 Proposed anti-cannibalism toxicity test container

The difficulty of toxicity test in crustacean larvae is to control the cannibalistic behaviour. Although a number of preys will be added to minimized the cannibalism and prevent starvation, attack by another larvae still occurs. A researcher would be confused if the summation of survivors and remains of dead larvae at a given endpoint does not balance the total number of exposers at the beginning if the same problem had taken place on other replicates. Generally, there are no partitions within experimental container. The issue is how to contain a number of organisms within a container without cannibalism and experience the same medium. A partition made up from fine mesh (mosquito larval-net is alternatively recommended) for walls of which, and coarser mesh for lining of bottom in order to sieve fecal pellet through it.

More systematically, a model of testing container for solving this problem is proposed schematically in Fig. 5.1. The container can personally make up from a clear plastic sheet by thermal figuration technique. Available material such as plaster of Paris or plywood can be applied for setting up the pattern. An unit contains a number of wells (at least 10 wells), the shape and dimension of well depends on the organism used in bioassay. Each well is linked with a series of slit along the bottom edge of the wells, allowing media transfer and circulation wholly throughout the system. Additional patches of pores on wall of the wells could be done to enhance water circulation. Alternatively, ice cube trays can be modified for this purpose, clear polystyrene type is prefer to dull polypropylene.

As regards aeration, in case air-stone does not fit the well dimension or Tygon tube is not appropriate, other type of aeration may be necessary to maintain sufficient oxygen in the media. A propeller connected with minimotor have to be installed on the center of basement basin with slightly conal in shape. This alternative is recommended because the direct aeration by air-stone or rubber siphon are relatively less efficient. Although air-stone can provide large amount of small bubbles with potential oxygen source, but exceed turbulence may effect on prey capture of larvae. Furthermore, TBTO is probably adsorbed on air-stone and decrease in content.

For Tygon tube, the relatively very big bubble can not supply sufficient dissolved oxygen, and provide turbulence as high as air-stone. Alternatively, a small agitator can produce constant water movement, provide water circulation, and enhance oxygenation through diffusion. Water renewal will be executed by gravitational force through the series of slit. The solution together with fecal pellet and undesirable material would be continuous removed from the wells through the slits and settle down on the center of conal basin. A circular groove around the aerator functions as effluent way control by a valve which will be turn off during 24 h of exposure.

5.2.2 Research recommended to figure out the status of TBT in Thai waters

Even though TBT anti-foulant is an worldwide environmental concerns, there are insufficient information associated with these compounds and related products to evaluate the overview status in Thailand. Many foreign studies are available on its acute and subacute toxicity in aquatic organisms on which few of those may be applied in some native species. Unfortunately, only one survey was conducted in Thailand for TBT contamination in environmental compartments: sediment (Kan-atireklap *et al.*, 1997a) and mussel (Kan-atireklap *et al.*, 1997b). However, the high incidence of imposex in sublittoral and littoral gastropods in Inner and South part of the Gulf of Thailand (Swennen *et al.*, 1997) reflects the environmental concerns in open sea areas. Although the distribution of TBT in riverine and estuarine systems are questionable, information given by an agent of Southeast Asia Fisheries Development Center (SEAFDEC) confirms the use of TBT anti-fouling paint, tributyltinmethacrylate/ methylmethacrylate copolymers which manufactured by Chugoku company (Sakya Pradit and Siriporn Pradit Pradit, personal communication, 15 October 2000) in Samut Prakarn area. Data gaps in TBT contamination in Thai waters are summarized in Table 5.1.

Several research recommendations follow from the datagaps presented in Table 5.1 are proposed:

(1) Determination of TT in Thai rivers, estuaries, and sea to form the contamination profile to assess the status in Thailand. Moreover, TBT levels in sediments and biota should be also performed.

(2) A list of anti-foulants available in Thailand should be inventoried, including the demand per annum. Anti-fouling coated boats and vessels should be registered and restricted. Such information required on release sources (e.g. hull areas and active ingredients) are served as data for calculating TBT flux in Thai waters.

(3) More data on persistence of butyltin species in aquatic compartments; kinetics of assimilation, metabolism, detoxification and bioaccumulation in biota; reservoirs in sediments (sedimentation rate and mixing effects such as bioturbation/storm mixing/current effects)

(4) Determination of TBT toxicity to native aquatic species to compare the sensitivities and select appropriate bioindicators. *M. rcsenbergii* larvae and youngs have potential to use as sentinels, respectively in estuaries and freshwaters.

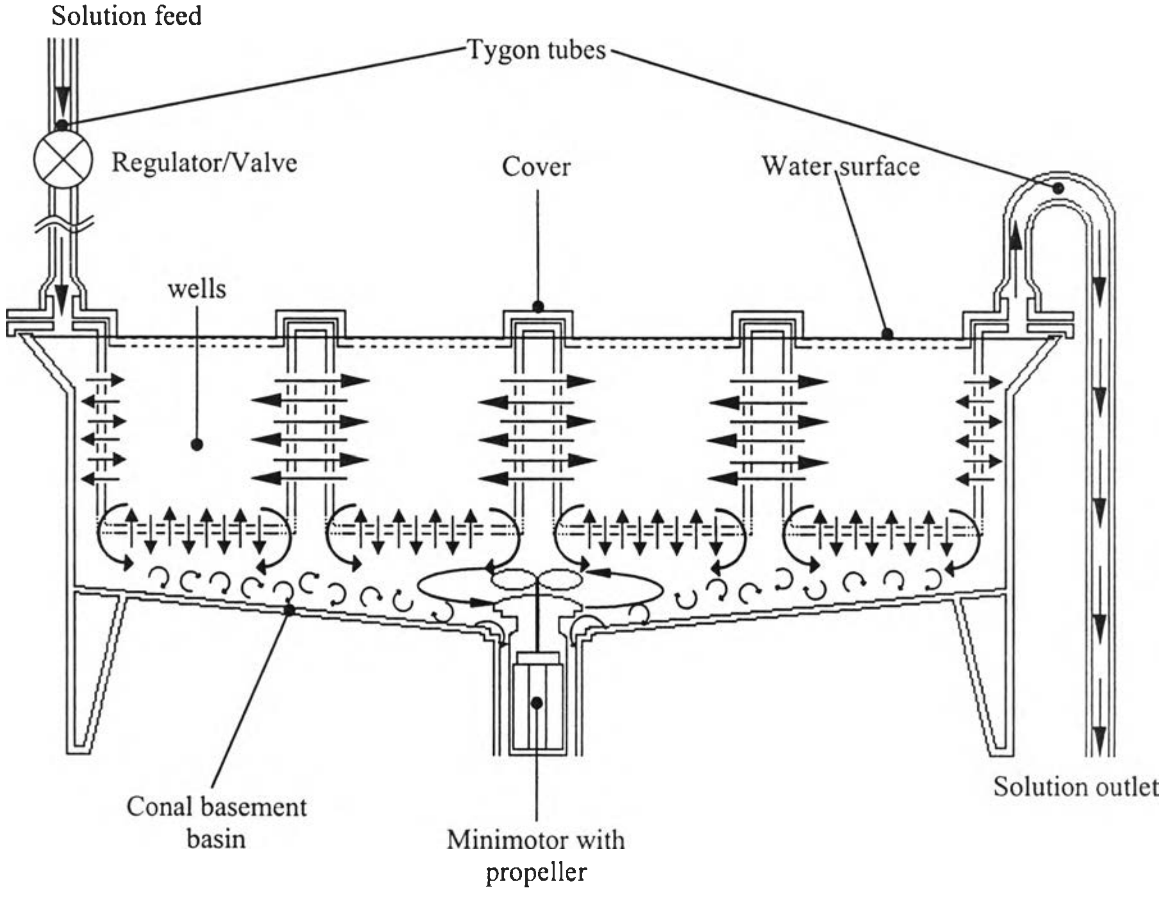

Fig. 5.1 Schematic diagram of proposed anti-cannibalism toxicity test container. Straight and curve arrows indicate water circulation, small circular arrows represent sediment transport.

Table 5.1 Datagaps in TBT contamination status in Thai waters

Topic/Issue	Sea	Estuary	River/Lake
Ambient concentration			
• Water	NA	NA	NA
• Sediment	4-450 ng g^{-1} dry wt as TBT (Kan-atirektap *et al.*, 1997a)		
• Biota	Silver pomfret, *Pampus argenteus* 1.3 ng g^{-1} wet wt, Indian mackerel, *Rastrelliger kanagurta* 2.1 ng g^{-1} wet wt, sea perch, *Lates calcarifer* 13 ng g^{-1} wet wt (Kannan *et al.*, 1995); Green mussel, *Perna viridis* 4-800 ng g^{-1} wet wt (Kan-atirektap *et al.*, 1997b)	NA	NA
Contamination sources			
• Annual anti-fouling paint demand	NA	NA	NA
• Number of vessels which applied anti-fouling paint, coated surface on hull, and types of applied paints	NA	NA	NA
Toxicity			
• Acute	Black tiger prawn, *Peneaus monodon* (Songkrit Papakdee, 1995); sea bass, *Lates calcarifer* (Warintorn Manosittisak, 1996)	Giant freshwater prawn *Macrobrachium rosenbergii* (from this study)	Giant freshwater prawn *Macrobrachium rosenbergii* (from this study)

Table 5.1 cont.

Topic/Issue	Sea	Estuary	River/Lake
• Subacute	Sea bass, *L. calcarifer* (Warintorn Manosittisak, 1996)	Giant freshwater prawn *M. rosenbergii* (from this study)	Giant freshwater prawn *M. rosenbergii* (from this study)
• Chronic	NA	NA	NA
• Threshold	NA	NA	NA
• Safety levels/ NOEC	NA	NA	NA
• Risky/affected local species	The Gulf of Thailand: (Gastopoda, Prosobranchia) *Babylonia areota*, *Burs rana*, *Cymbiola nobilis*, *Harpa articularis*, *Hemifusus ternetanus*, *Melo melo*, *Murex* sp., *M. trapa*, *M.tribulis*, *Nassarius livescens*, *N. stolatus*. *Phalium bisulcatum*, *P. glaucum* *Siphonalia varicosa*, *Thais hippocastaneum*, *T. Lacera*, *Volema cochlidium* (Kannan *et al*., 1997) Phuket Island: (Gastopoda, Prosobranchia) *Morula muvisa* (Bech, 1999)	NA	NA
Control/regulation	Consequent from foreign restriction	Consequent from foreign restriction	Consequent from foreign restriction
Monitoring	The Gulf of Thailand (Swennen *et al*., 1997) and Phuket (Bech, 1999)	NA	NA

5.2.3 Feasibility of *Macrobrachium rosenbergii* for using as standard testing animal

To assess an intrinsic impact of pollutants on aquatic ecosystem, understanding on effects of the stressors on life cycle of aquatic species is necessary. Alteration of population dynamics and/or health of affected organisms could initiate chain of impact throughout the niche.

Immature overview pertaining to the aspect stated above rises difficulty and uncertainty in environmental impact assessment. Critically, incomplete mitigation measures are finally shot by that mistake. Information which addresses these question is the approach to minimize the false evaluations and recommendations. For this purpose, representative species should be tested for the pollutants' effects throughout its life cycle. Unfortunately, due to limited budget and timeframe, most studies are focused on toxic effects in part of life span within a short period of exposure. Especially, effects on early life stage which is the most sensitive duration is scarce. In addition, effects on reproductive capability are very interesting topics as well. Thus, researches regarding to those issues should be promoted and performed.

M. rosenbergii is a considerable and potential species for serving as standard testing animal, larvae for brackish, and young prawns for freshwater environments. The feasibility is evaluated by following criteria presented in Table 5.2.

Table 5.2 Feasibility of *Macrobrachium rosenbergii* for using as standard testing animal

Criteria	Attribute	Comments	Feasibility
Availability	Whole year round	Easy and convenient to available in a required size and amount from prawn farms.	Yes
Intrinsic important	Economic species	Population impact and natural stock alternation could be linked to socio-economic impact.	Yes
Consistency of response	Acceptable variation	Standard method should be established for another sets of experiments.	OK
Culturing/Rearing in laboratory	Laboratory scale is enable.	Brood stock can be provided and maintained in laboratory to serve experiments.	Yes
Distribution	Throughout Thailand	Its widespread natural habitats in many Thai rivers makes it appropriate to utilize as representative for freshwater species.	Yes
Life cycle	Consumes about 6 months for eggs to become sexually mature prawns (~10 cm from rostrum to telson).	It is possible to design an experiment throughout a life cycle within one year.	Yes
Sensitivity	Hatchability is affected at 3.5 μg TBTO l^{-1} for 16 days of exposure. Lowest observable effect concentration at 0.6 μg TBTO l^{-1} induces growth retardation in larvae.	Comparisons to other native species are recommended.	?

Table 5.2 cont.

Criteria	Attribute	Comments	Feasibility
Sensitivity (cont.)	Fifty percent mortality occurs in embryos ranging from 539-699 µg TBTO l^{-1} after 96 h of exposure LC_{50}s for early stage larvae are 5.8-7.7 and 10.3-14.3 µg TBTO l^{-1} for 24 and 48 h of exposure, respectively.	Comparisons to other native species are recommended.	?
Size	About 2-8 mm for larval stage; 5 cm for 2 month-old juveniles; 10 cm for sexually mature youngs	Acceptable sizes from larval to adult are obtained.	Yes