Chapter 4

Results and Discussion

4.1 Acute toxicity of TBTO

4.1.1 Embryo toxicity tests

Pretests

On the range finding test, 3 d-old embryos were treated with nine concentrations between 0.5 to 128 µg TBTO l^{-1} in arithmetic series whose survived and still developed at the maximum concentration. A slightly advanced develops than those in solvent control was noted in third day of exposure. The new range was therefore set up for 25, 50, 100, 200, and 400 µg TBTO l^{-1}. At 96 h of exposure, no embryos were killed at 25 and 50 µg TBTO l^{-1} (Fig. and Table 4.1), some died at 100, 200 and 400 µg TBTO l^{-1}. Otherwise, no obvious abnormalities could be detected under microscopic examination among survivors at all concentration.

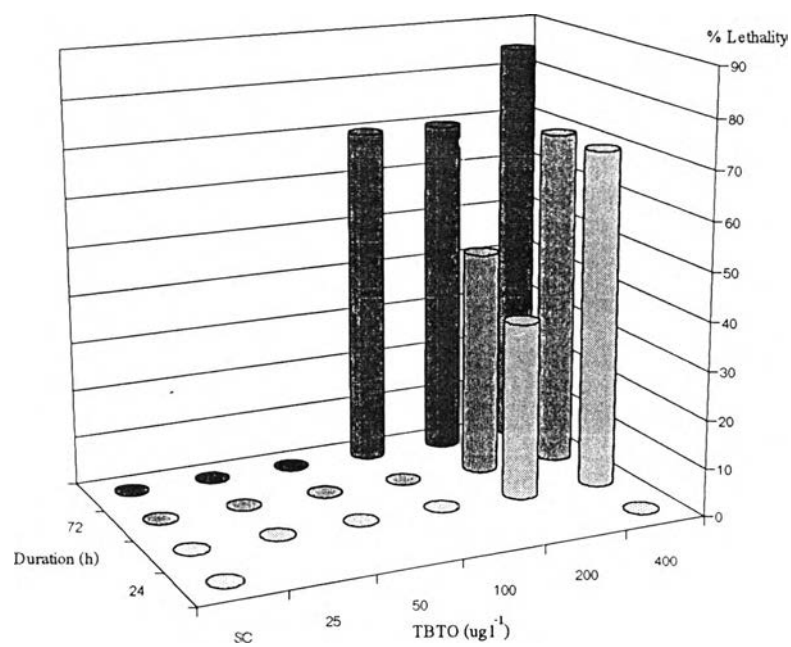


Fig. 4.1 Mean percent lethality at 24, 48, 72, and 96 h for 3 day-old *Macrobrachium rosenbergii* embyros exposed to different concentrations of TBTO (2nd range finding test)

Table 4.1 Mean percent lethality at 24, 48, 72, and 96 h for 3 day-old *Macrobrachium rosenbergii* embryos exposed to different concentrations of TBTO (2nd range finding test)

Duration	TBTO ($\mu g\ l^{-1}$)					
(h)	SC	25	50	100	200	400
24	0	-	-	-	-	0
48	0	0	0	0	36.7±5.6	70±30
72	0	0	0	0	46.7±11.5	70±14.1
96	0	0	0	25±7.1	46.7±11.5	85±7.1

n = 10 embryos per treatment; SC, solvent control; -, not determined

In third trial, embryos were treated at concentrations 200, 250, 300, 350, and 400 µg TBTO l^{-1} for 96 h. The result shows that after 24 h, lethality was not obviously increased with time until the end of 96 h (Fig. and Table 4.2). Lethality did not changed at 48 and 72 h of exposure. At 96 h, the highest concentration (400 µg TBTO l^{-1}) was firstly examined for lethality, which did not meet 50%. So, other concentrations were not checked and discarded. These testing concentrations were not the appropriate range.

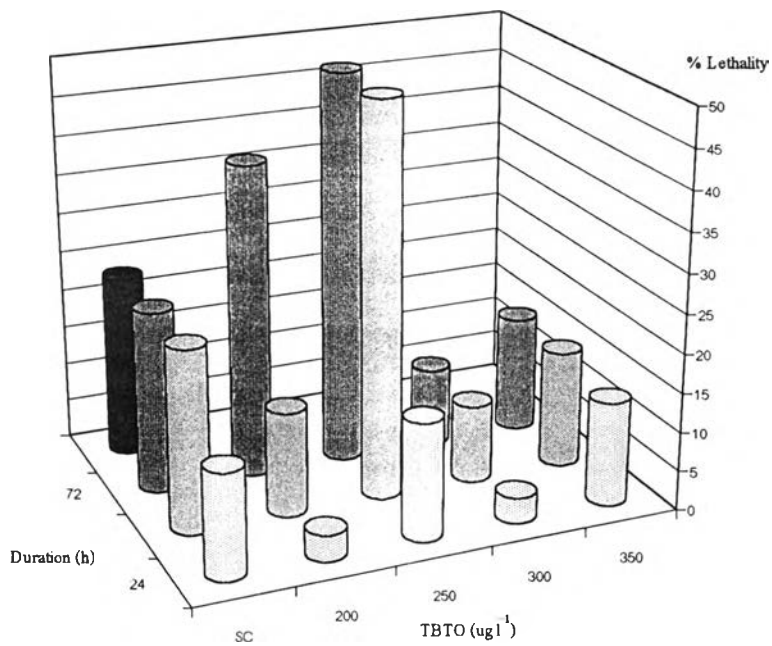


Fig. 4.2 Mean percent lethality at 24, 48, 72, and 96 h for 3 day-old *Macrobrachium rosenbergii* embryos exposed to different concentrations of TBTO (3rd range finding test)

Table 4.2 Mean percent lethality at 24, 48, 72, and 96 h for 3 day-old *Macrobrachium rosenbergii* embryos exposed to different concentrations of TBTO (3rd range finding test)

Duration	TBTO ($\mu g\ l^{-1}$)					
(h)	SC	200	250	300	350	400
24	13.3±5.8	3.3±5.8	15±7.1	3.3±5.8	13.3±5.8	13.3±15.3
48	23.3±5.8	13.3±15.3	50±0	10±10	15±7.1	30±0
72	23.3±5.8	40±0	50±0	10±10	15±7.1	35±7.1
96	23.3±5.8	-	-	-	-	40±14.1

n = 10 embryos per treatment; SC, solvent control; -, not determined

No inhibition of developmental rate was detected in all concentration. It is assumed that the mortality occurred in third trial not caused by TBTO, but possibly by injury during egg detaching from berried prawn brood chamber using soft brush. So the detached eggs were retained in container by 24 h before test, the dead ones were discarded. In addition, health and sensitivity of individuals to TBTO might be a wide variety for this crop.

Because of the lack of sensitivity of the embryos, the new range finding test ten embryos were treated with 500, 1,000, and 5,000 µg TBTO l^{-1}. The range was started at 500 µg TBTO l^{-1} for easy preparation from 1 mg TBTO l^{-1} (stock solution II). The result showed that all exposed embryos were killed within 24 h at 5,000 µg TBTO l^{-1} (Table and Fig. 4.3), the actual definitive test was then run at 250, 350, 500, 700, and 1000 µg l^{-1}.

Table 4.3 Mean percent lethality at 24, 48, 72, and 96 h for 10 day-old *Macrobrachium rosenbergii* embryos exposed to different concentrations of TBTO (4th range finding test)

Duration	TBTO ($\mu g\ l^{-1}$)			
(h)	SC	500	1000	5000
24	0	53.3±15.3	66.7±5.8	100
48	3.3±5.8	66.7±15.3	80±17.3	-
72	3.3±5.8	86.7±15.3	85±7.1	-
96	6.7±5.8	85±7.1	100	-

n = 10 embryos per treatment; SC, solvent control; - terminated replicate(s)

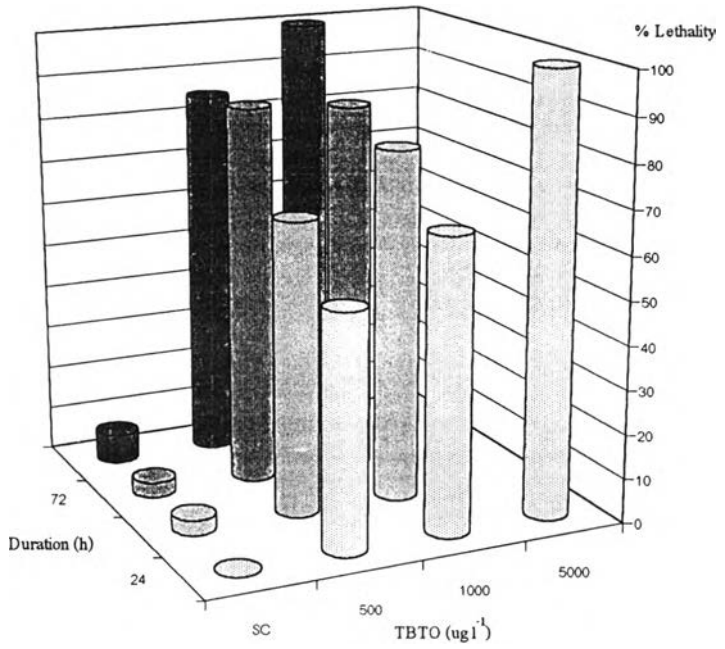


Fig. 4.3 Mean percent Lethality at 24, 48, 72, and 96 h for 10 day-old *Macrobrachium rosenbergii* embryos exposed to different concentrations of TBTO (4th range finding test)

Actual tests

Mean percent lethality and corresponding LC_{50}s with their 95% confidential intervals for both selected stages of embryos are presented in Table 4.4. The 96 h LC_{50}s for early stage embryos was 583 µg l^{-1} (499-693), which was lower than that for late stage (d12) embryos, 699 µg l^{-1} (497-1786). Although a chain of 96 h acute toxicity were not performed to cover the 19 days of incubation period, the representative LC_{50}s of both the early and late stage embryos tended to increase with the progress of embryonic development. Thus, the sensitivity to TBTO decreased with higher age of embryos.

Unfortunately, the scarce papers with regard to the embryo toxic of TBTO or other TBT compounds on crustaceans limited a comparison of sensitivity to TBTO with this study. The existing available data is the work on embryo toxicity conducted in the other taxa. As in the study on medaka or red killifish *Orizias lapites* (Bentivegna and Piatkowski, 1998), the 96 h LC_{50}s ranged from 159 nM (equivalent to 55 µg l^{-1}) for 0 day-old embryos to 340 nM (equivalent to 119 µg l^{-1}) for 5 day-old embryos indicated lower tolerance than *M. rosenbergii* embryos (539 µg l^{-1} for early stage and 699 µg l^{-1} for late stage). It obviously indicated that *M. rosenbergii* embryos were less sensitive.

Furthermore, Bentivegna and Piatkowski (1998) applied TBTO to the test animals once without renewal which allowed medaka embryos to recover with time, while this experimental condition was static daily renewal design. In addition, the different biochemical structure between the chorion of medaka and egg membrane of *M. rosenbergii* might be resulted in different tolerance to TBTO. The present study agrees with the work in medaka embryos and suggests that TBTO toxicity was age dependent: the sensitivity to TBTO decreased with increasing developmental stage.

Table 4.4 LC_{50}s and mean percent lethality at 48, 72, and 96 h for *Macrobrachium rosenbergii* embryos exposed to TBTO on different stages of development

Stage	Duration	LC_{50} ($\mu g\ l^{-1}$)	TBTO ($\mu g\ l^{-1}$)					
		[95%CI]	SC	250	350	500	700	1000
Early (d1-5) $n = 15$	96	583 [499-693]	0	3.3±4.7	13.3±9.4	40±18.9	46.7±28.3	100
Late (d12-16) $n = 12$	24	-	0	0	0	8.3±0	8.3±0	0
	48	1472 [877-640226]	0	0	8.3±0	25±0	33.3±8.3	33.3±0
	72	1259 [759-2642324]	0	4.2±5.9	16.7±0	25±0	26.7±0	30±4.7
	96	699 [497-1786]	0	4.2±5.9	37.5±5.9	41.7±8.3	50±8.3	58.3±8.3

C, control; SC, solvent control; -, not calculated

4.1.2 Larval toxicity tests

Pretests

First trial was initiated in 4th-stage larvae over three days period, no signal of positive dose related to mortality data (Fig.4.4 and Table 4.5). High mortality occurred despite on sublethal concentration (0.2 and 0.4 $\mu g\ l^{-1}$) at 72 h of exposure, while relatively lower mortality appeared at 3 $\mu g\ l^{-1}$ which is an order-higher concentration. It is assumed that there are other causes than TBTO, such as health of larvae or handling technique, which killed these larvae.

In second range finding test, 5 concentrations of 3, 6, 12, 24, and 48 $\mu g\ l^{-1}$ were conducted to meet appropriate lethal concentration range. The test was performed in 1st, 2nd, and 6th-stage larvae, the results showed that most of larvae were killed between 6 and 24 $\mu g\ l^{-1}$ at 24 h, and start at 3 $\mu g\ l^{-1}$ for 48 h lethality (Fig. 4.5 and Table 4.6). Therefore, the actual range was set in arithmetic series

with a factor of 1.5 for five concentration values. The round-up values were 4, 6, 9, 14, and 20 μg l^{-1} and were used for acute lethal toxicity test.

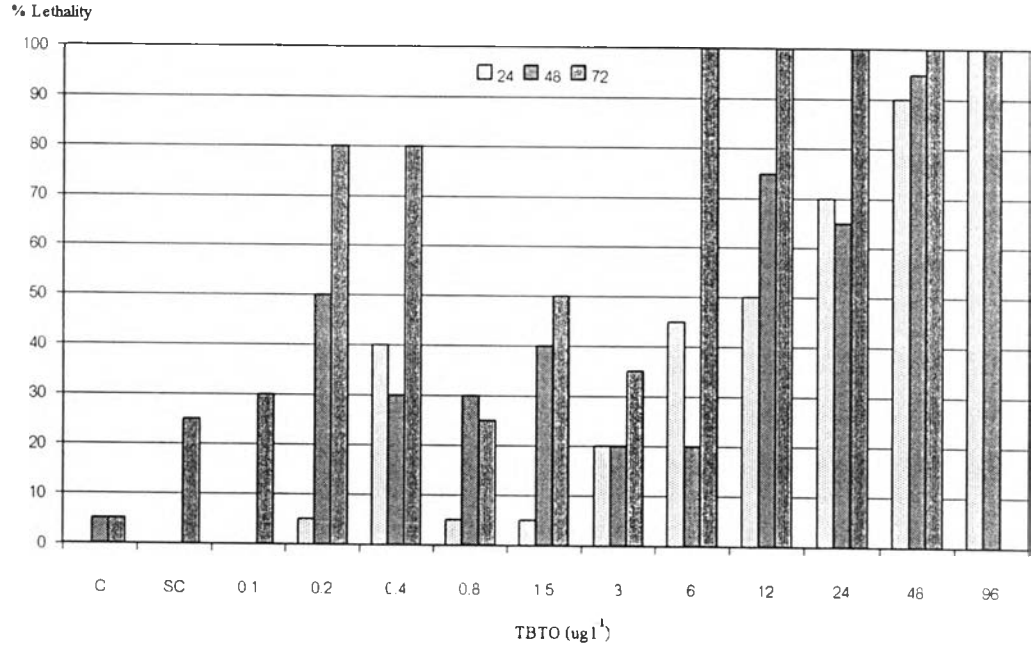


Fig. 4.4 Mean percent lethality at 24, 48, and 72 h for 4th-stage *Macrobrachium rosenbergii* larvae exposed to different concentrations of TBTO (first trial)

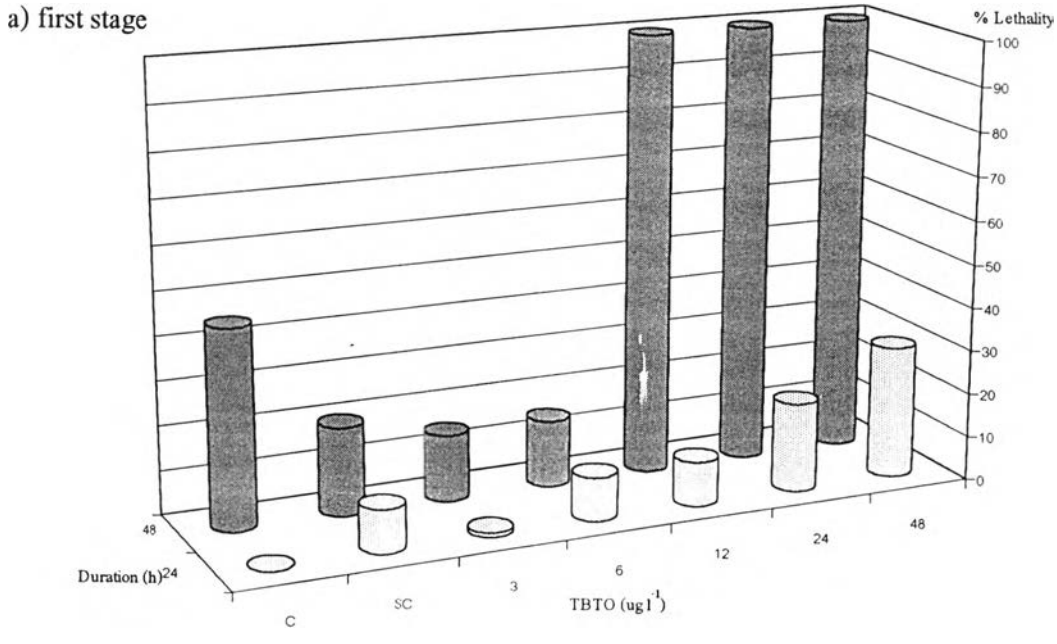
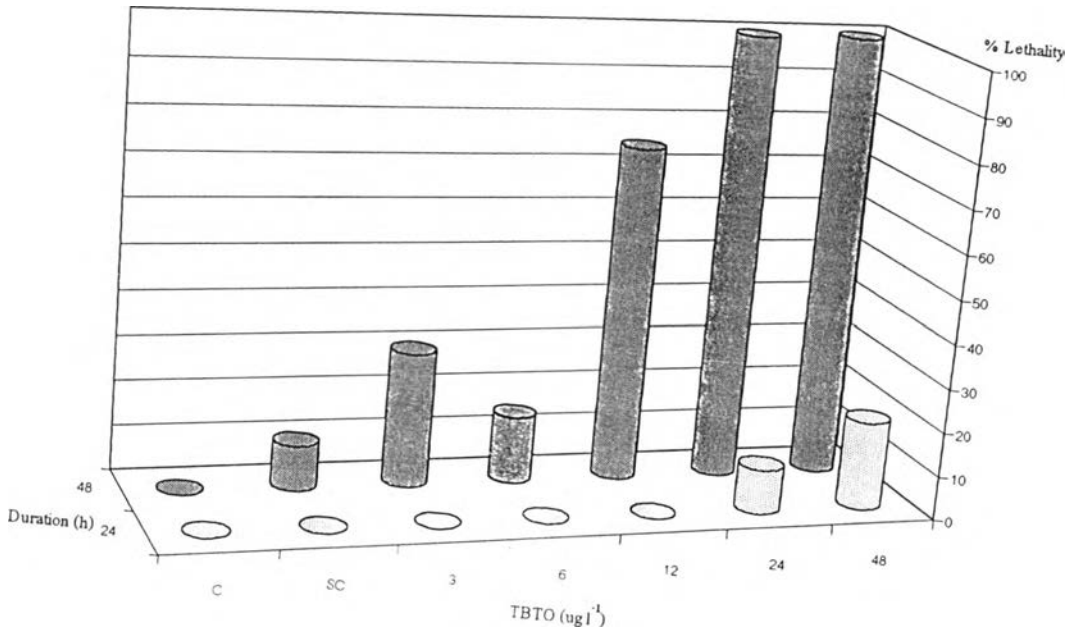


Fig. 4.5 Lethality at 24 and 48 h for 1st, 2nd, and 6th-stage *Macrobrachium rosenbergii* larvae exposed to different concentrations of TBTO (second trial)

b)second stage



c) sixth stage

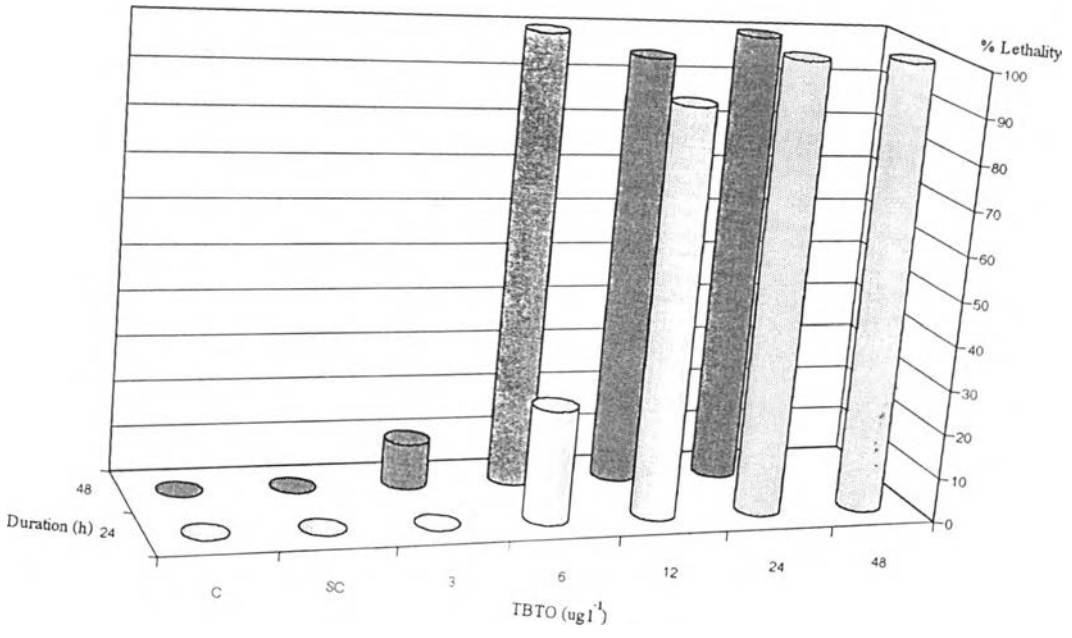


Fig. 4.5 cont.

Table 4.5 Mean percent lethality at 24, 48, and 72 h for 4th-stage *Macrobrachium rosenbergii* larvae exposed to different concentrations of TBTO (first trial)

Duration	TBTO ($\mu g\ l^{-1}$)												
(h)	C	SC	0.1	0.2	0.4	0.8	1.5	3	6	12	24	48	96
24	0	0	0	13±15	40±0	23±32	13±15	17±5.8	37±15	37±15	53±5.8	40±0	100
48	13±15	3.3±5.8	6.7±12	50±10	43±15	30±30	33±12	17±5.8	63±21	73±15	87±15	97±5.8	-
72	13±15	17±15	40±17	70±17	70±17	35±25	67±31	57±38	97±5.8	100	100	100	-

C, control; SC, solvent control; -, terminated replicated(s)

Table 4.6 Mean percent lethality at 24 and 48 h for 1st, 2nd, and 6th-stage *Macrobrachium rosenbergii* larvae exposed to different concentrations of TBTO (second trial)

Stages	Duration	TBTO ($\mu g\ l^{-1}$)						
	(h)	C	SC	3	6	12	24	48
1	24	0	10±10	3.3±5.8	6.7±5.8	10±10	20±10	30±10
	48	45±7.1	23±5.8	23±15	15±7.1	100	100	100
2	24	3.3±5.8	3.3±5.8	0	3.3±5.8	3.3±5.8	13±5.8	47±47
	48	3.3±5.8	6.7±5.8	30±0	15±7.1	83±15	97±5.8	100
6	24	0	0	0	37±21	73±31	97±5.8	100
	48	0	0	10±10	97±58	95±7.1	100	

C, control; SC, solvent control; terminated replicated(s)

Actual tests

In larvae, lethality at 24 and 48 h larvae exposed to TBTO at different concentrations is presented in Table 4.7. Twenty four h-LC_{50}s for first to eighth stage larvae, and second to sixth stages for 48 h are indicated in Table 4.8 and 4.9, respectively. The 24-h LC_{50}s appeared to be not different among first to eighth stages. The 24 h LC_{50}s were between 10.0 μg l^{-1} (6.7-16.4; 3rd stage) to 14.2 μ g l^{-1} (11.9-16.7; 8th stage).

For 48 h LC_{50}s, a range of 5.3 to 7.7 μg l^{-1} were obtained. There manifest to be not difference in LC_{50}s among stages of the larvae. The 48 h LC_{50}s ranging from 5.3 (0.2-8.5; 5th stage) to 7.7 (5.8-10.1; 6th stage).

For 24 h of exposure, the data obtained from this study are comparable to previous studies. In American lobsters *Homarus americanus*, were totally killed at a concentration of 7.6 μg l^{-1} (Laughlin and French, 1980). Recent studies were conducted in two species of penaeid shrimps, *P. monodon* (Songkrit Prapakdee, 1996) and zebra shrimps *P. japonicus* (Lignot *et al.*, 1998), 24 h LC_{50}s ranging from 0.89 (nauplius) to 3.39 (PL6) μg l^{-1} was reported for the former, and the range of 1 to 5.3 μg l^{-1} was observed in zoea3 and mysis3, respectively in the later. Larval *M. rosenbergii* distinctively display less sensitivity to TBTO than others.

In the period of 48 h of exposure, LC_{50}s were ranged between 0.7 μg l^{-1} in mysis1 of *P. japonicus* and 1.4 μg l^{-1} in mysis3 (Lignot *et al.*, 1998). As regards planktonic crustacean, the 48 h LC_{50}s were found to be 0.47 and 0.24 μg l^{-1}, respectively in marine copepods *Acatia tonsa* at salinity of 18‰ and 28‰ (Kusk and Petersen, 1997). For the present study, 48 h LC_{50}s for larval *M. rosenbergii* are between 5.8 and 7.7 μg l^{-1}, It is clear that *M. rosenbergii* larvae have relatively high tolerance to TBTO comparing to planktonic crustacean.

In addition, the disposing surface area is possibly a factor of the relative sensitivity to TBTO for these shrimp. *M. rosenbergii* larvae have relatively short and tough body compared to slender body of paeneid shrimp larvae. It is intuitively therefore, *M. rosenbergii* larvae have lower disposing surface area than those larval penaeids, and showed higher tolerance to TBTO concentraions in the same exposure duration.

M. rosenbergii in different stages of life cycle, the embryos show higher tolerance than those larvae by 90 to 120 times of median lethal concentration. The different order of magnitude obviously indicated that embryos were less sensitive to TBTO than larvae. The lack of sensitivity might due to the uptake rate. Compared to hatched larvae, the egg membrane might have protected the embryos like the chorion of medaka embryos (Bentivegna and Piatkowski, 1998). Although the very higher doses were applied to the embryos, they can survive for longer exposure duration. By contrast, TBTO could directly attack to the target organ at gill of larvae and epipodites also, as histopathological effects described by Lignot *et al.* (1998). The relative less sensitive to TBTO of early stage of *M. rosenbergii* larvae found in this study might be due to its euryhaline characteristics, the significant higher tolerance in the first sixth stages confirmed its excellent adaptation to wide alteration of environmental condition from freshwater to estuary. However, There are no obvious increasing trend of LC_{50}s with progressive development as found in penaeid shrimps (Lignot *et al.*, 1998; Songkrit Prapakdee, 1996).

Table 4.7 LC_{50}s and mean percent lethality at 24 h for *Macrobrachium rosenbergii* larvae exposed to TBTO on first to eighth stage of development

Stage	LC_{50} ($\mu g\ l^{-1}$)	TBTO ($\mu g\ l^{-1}$)											
	[95%CI]	SC	3	4	6	9	12	14	20	24	30	45	48
1	10.9 [6.8-13.7]	0	-	-	-	25±7.1	-	85±21	90±14	-	95±7.1	100	-
2	12.5 [9.2-16.4]	0	-	-	13±5.8	35±7.1	-	40±10	90±14	-	90±10	-	-
3	10.0 [6.7-16.4]	0	-	20±0	23±5.8	50±10	-	53±45	85±7.1	-	-	-	-
4	10.6 [5.4-19.2]	0	17±5.8	-	37±15	-	53±5.8	-	-	67±5.8	-	-	90±0
5	12.0 [9.7-15.2]	0	-	0	10±0	10±0	-	85±21	80±14	-	-	-	-
6	12.0 [8.7-20.8]	0	-	10±0	25±7.1	30±0	-	50±0	80±14	-	-	-	-
7	10.3 [8.7-12.2]	0	-	-	0	30±0	-	90±0	100	-	100	-	-
8	14.2 [11.9-16.7]	0	-	-	0	5±7.1	-	40±14	95±7.1	-	100	-	-

SC, solvent control; -, not tested

Table 4.8 LC_{50}s and mean percent lethality at 48 h for *Macrobrachium rosenbergii* larvae exposed to TBTO on second to sixth stage of development

Stage	LC_{50} ($\mu g\ l^{-1}$)	TBTO ($\mu g\ l^{-1}$)											
	[95%CI]	SC	3	4	6	9	12	14	20	24	30	45	48
2	6.4 [2.3-8.9]	0	-	-	60±0	50±10	-	85±7.1	97±5.8	-	100	-	-
3	6.9 [3.3-10.1]	0	-	45±7.1	30±0	55±7.1	-	65±21	100	-	-	-	-
4	6.2 [3.4-9.5]	0	13±5.8	-	65±7.1	-	70±20	-	-	93±5.8	-	-	-
5	5.3 [0.2-8.5]	0	-	33±5.8	63±5.8	63±5.8	-	93±5.8	73±12	-	-	-	-
6	7.7 [5.8-10.1]	0	0	-	25±7.1	-	90±14	-	-	100	-	-	100

SC, solvent control; -, not tested

4.2 Subacute toxicity of TBTO

4.2.1 Embryotoxicity tests

The changes of embryo during development are illustrated in Fig. 4.6, the age of embryo cannot be exactly determined or quantified by morphological characteristics. Keystone recognitions are the appearance of optic rudiment on seventh day and the beating heart on eighth day.

Due to the difficulty of embryonic development stage classification, although the investigation was performed stereoscopically, the effects of TBTO on developmental rate could only checked for eye vesicle formation between day 7 and 9 after fertilization. It was found that under this experimental condition the occurrence of embryos' eyes was detected faster than those attached in the brood chamber of the mother prawn for one day. This effect also found in the control and solvent control. This consequence affected hatch time, 9% of embryos in solvent control started hatching one day before it took place in the berried prawn. Hatching was relatively low at about 1% for 3.5 for 14.0 $\mu g\ l^{-1}$. Hatchability shows a concentration-related manner (Fig. 4.7 and Table 4.9), hatching success was 63% for solvent control, 40% for 3.5 $\mu g\ l^{-1}$, 15% for 7.0 $\mu g\ l^{-1}$, 5.2% for 14.0 $\mu g\ l^{-1}$, 1.0% for 28 $\mu g\ l^{-1}$ and no survivor left at the highest concentration of 57.0 $\mu g\ l^{-1}$. No malformation of hatched larvae was detected stereoscopically at any concentrations, only one larva died after hatching on 16th day of exposure. Otherwise, the dead larva did not display any malformation characteristic.

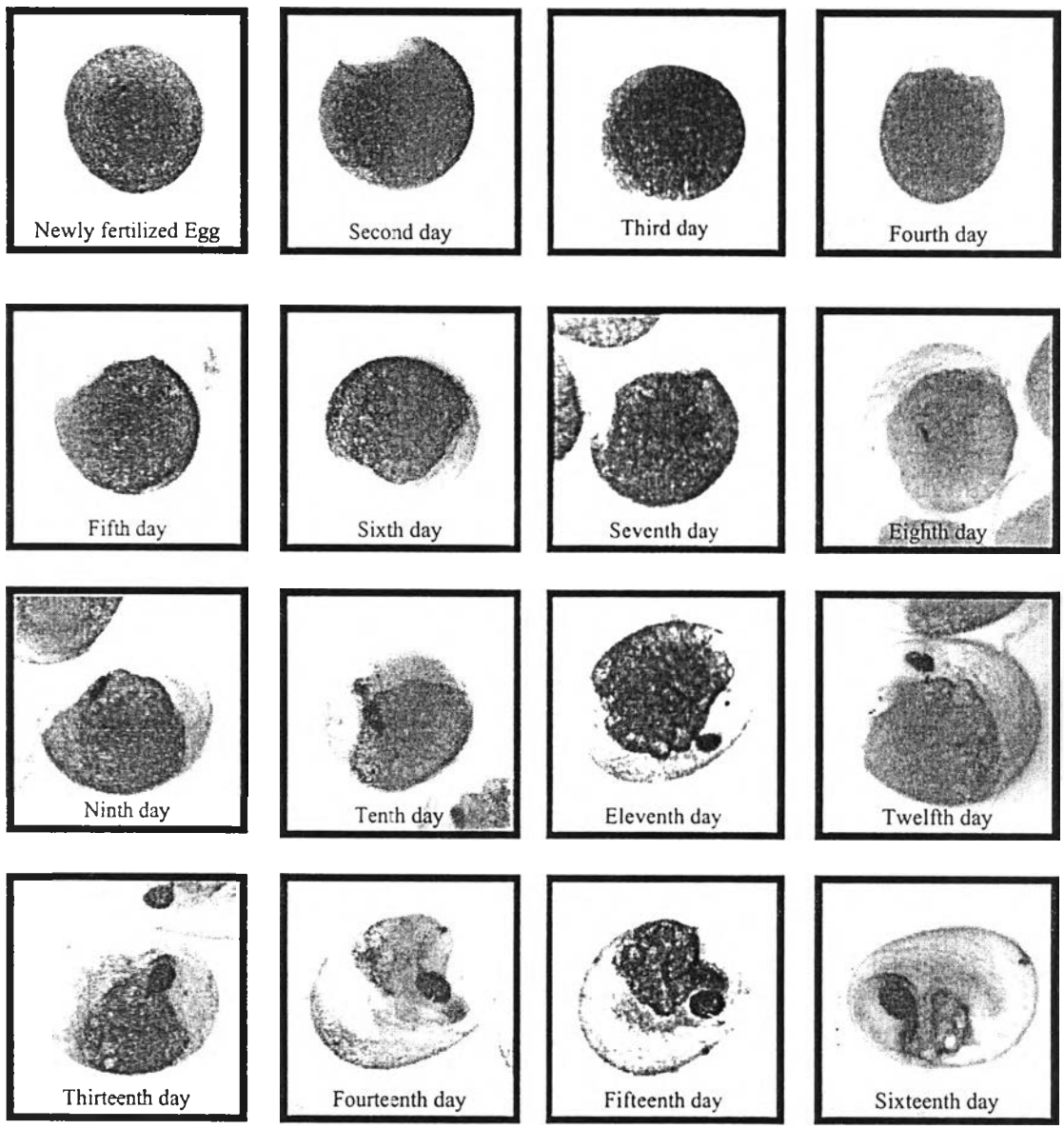


Fig. 4.6 Embryonic development of *Macrobrachium rosenbergii* de Man (x 40 magnified)

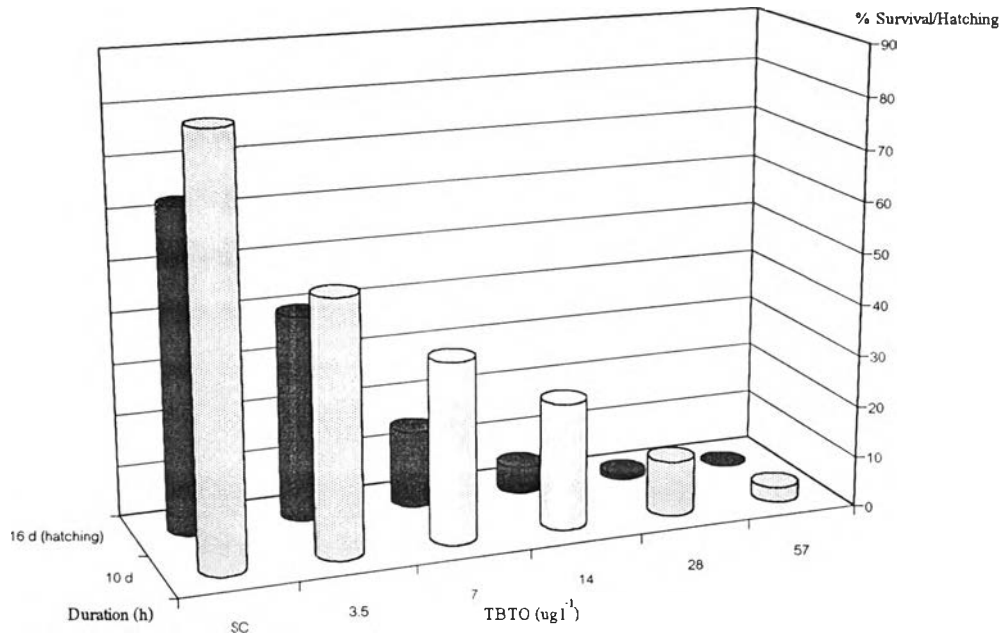


Fig. 4.7 Percent survival on 10th day and percent hatching on 16th day of exposure in *Macrobrachium rosenbergii* treated with TBTO

Table 4.9 Percent survival on 10th day and percent hatching on 16th day of exposure in *Macrobrachium rosenbergii* treated with TBTO

Day of exposure	TBTO ($\mu g\ l^{-1}$)					
	SC	3.5	7	14	28	57
10 d	81.82 (33)	49.45 (91)	35.17 (80)	24.68 (77)	10.78 (102)	2.91 (103)
16 d (hatching)	62.96 (27)	40.00 (20)	15.00 (80)	5.20 (77)	0.98 (102)	0.00 (103)

SC, solvent control
Numbers in parenthesis indicate total embryos for calculation

4.2.2 Larval toxicity tests

Pretests

Growth retardation by TBTO at sublethal doses was initiated using 3rd, 4th, and 5th-stage larvae. Two different stages of survivors could be observed in each experimental unit. Unfortunately, the results were not seem in concentration manner (Table 4.10). The causes possibly are the health and its actual hatching time of individuals. The effects of TBTO on larval development can not demonstrated only by a short-term test within 48 h of transitional period.

According to this study, no malformation of hatched larvae treated with TBTO was observed by the end of incubation period at any concentrations. The results show that TBTO is embryotoxic, but is to be considered a non-teratogenic substance, characteristic behaviour of an embryo lethal compound. In contrast, it plays role as a teratogen for some vertebrate embryos by causing skeletal malformation (Pinkney *et al.*, 1990; Scodding, 1990; Fent, 1992) and ocular abnormalities (Weis, Weis *et al.*, 1987; Fent, 1992).

Actual tests

Percent stage composition of larvae treated with two dose of TBTO plus control and solvent control is shown in Table 4.11. The variation in stage composition started on ninth day in treated larvae, whereas consistent growth rates were observed in all larvae sampled from control and solvent control until the twelfth day. Variation was then started since thirteenth day for both control groups.

Subacute effects of TBTO on larvae caused retarded growth in a negative concentration-development relationship (Fig. 4.8 and Table 4.12). At the endpoint on day 30 of exposure, larvae in control and solvent control respectively reached tenth and ninth stage, whereas those treated with 0.6 or 1.2 µg TBTO l^{-1} still was about eight stage. There was no statistically significant difference between control and solvent control ($P < 0.05$). The difference between control or solvent control and treated groups were statistically significant ($P < 0.05$), while no significant difference was noted between 0.6 and 1.2 µg l^{-1} exposed groups. In addition, no malformed larvae were detected during daily staging by random sampling. Even though the growth rate between 0.6 and 1.2 µg l^{-1} treated larvae was not significantly different, it noted that the size of larvae exposed to the higher concentration of TBTO generally smaller or shorter than those exposed to the lower concentration in the same stage of development, especially in later instars. However, the differences were not quantified.

In this study, larval growth rate was decreased at concentrations, 0.6 and 1.2 µg l^{-1}. At endpoint of day 30, the treated larvae just reached eighth stage of development compared to tenth stage larvae in controls and solvent controls. It normally takes about 8 days for eight stage larvae to reached tenth stage, by taking about 4 days for each next stages (Uno and Kwon chin soo, 1969). These results agree with the test in zoeal mud crab *Rhithropanopeus harrissii* (Laughlin *et al.*, 1983)

which growth was significantly stunted at over 0.36 μg Sn l^{-1}. The metamorphosis time increased by 2 days at 10 μg TBTO Sn l^{-1} and by 6 days at 18 μg TBTS Sn l^{-1} compared to controls. Weis, Gottlieb *et al.* (1987) found that the leg regeneration and exuviation in fiddler crab *Uca pugilator* was delayed at 0.2 μg l^{-1} of TBTO, malformation in regenerated parts varied from 16.7% in control to 66.7% at a concentration of 10 μg TBTO l^{-1}. From previous studies, morphological anomalies induced by TBTO could only in crabs, no malformed shrimp affected by TBT was reported. As in this subacute toxicity test, no malformed larva was detected over the 30 days of exposure.

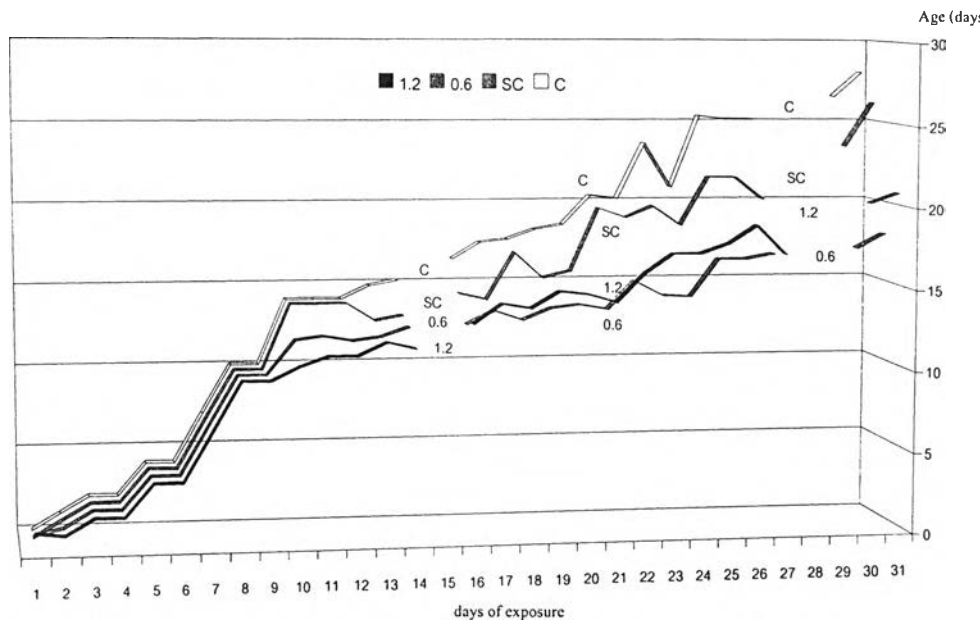


Fig. 4.8 Larval developmental rate after exposed to TBTO

Conversely, the abnormality affected by TBTO was reported in treated *P. monodon* on carapace, but the anomalies was not quantified (Songkrit Prapakdee, 1996). Nevertheless, it is difficult to diagnose since it can easily confused with growth retardation. Besides the teratogenic effects in shrimps including specific abnormalities have not been reported before, the author did not suggest the malfunction caused by the deformity. Moreover, the doubtful malformed carapace which only occurred in protozoea at 1.63 μg l^{-1} was not verified by morphometric technique. However, If TBTO had induced teratogenicity in the shrimps, the quantal data of malformed larvae in different concentrations of toxicant would be calculated for meadian teratogenic concentraion (TC_{50}). And the

teratogenic risk associated with TBTO may be estimated by the teratogenic index (TI) which is the ratio of LC_{50}/TC_{50} (Vismara *et al.*, 2000). TBTO would be considered as teratogen if the TI_{50} value is more than 0.5. Furthermore, the literature on TBTO toxicity in shrimp has indicated that it causes osmoregulation disruption at lethal and sublethal concentration (Lignot *et al*, 1998)

TBT levels that can be encountered in freshwaters and estuaries are usually in ng l^{-1} level. For instance, concentrations up to 26 ng l^{-1} TBT were found in five of six Swiss River. In some estuaries, as in Tianjin habour, China for example, the concentrations ranging from 15.69 to 41.78 ng l^{-1} TBT in waters (15.80-15.90‰ salinity) were reported by Ma *et al* (2000).

Based on present study, it can be considered that embryos and larval instars of *M. rosenbergii* (larvae may hatch in both fresh and brackish water under natural condition; Ling, 1969b) will not be affected by ambient TBT contamination levels reported in foreign countries.

Table 4.10 Percent stage composition and mean stage of 3rd, 4th, and 5th-stage *Macrobrachium rosenbergii* larvae exposed to different concentrations of TBTO for 48 h (pretests)

Stage	TBTO ($\mu g\ l^{-1}$)					
	SC	0.1	0.2	0.4	0.8	1.6
3	$80^{III}, 20^{IV}$ (3.2)	100^{III} (3.0)	$70^{III}, 30^{IV}$ (3.3)	$70^{III}, 30^{IV}$ (3.3)	$57^{III}, 43^{IV}$ (3.4)	-
	$50^{III}, 50^{IV}$ (3.5)	$80^{III}, 20^{IV}$ (3.2)	$78^{III}, 22^{IV}$ (3.2)	$80^{III}, 20^{IV}$ (3.2)	$89^{III}, 11^{IV}$ (3.1)	-
	$75^{III}, 25^{IV}$ (3.3)	100^{III} (3.0)	$80^{III}, 20^{IV}$ (3.2)	$80^{III}, 20^{IV}$ (3.2)	100^{III} (3.0)	-
4	$44^{IV}, 56^{V}$ (4.6)	$50^{IV}, 50^{V}$ (4.5)	100^{IV} (4.0)	$67^{IV}, 33^{V}$ (4.3)	$25^{IV}, 75^{V}$ (4.8)	$62.5^{IV}, 37.5^{V}$ (4.4)
	$30^{IV}, 70^{V}$ (4.7)	100^{IV} (4.0)	$67^{IV}, 33^{V}$ (4.3)	$14^{IV}, 86^{V}$ (4.7)	$87.5^{IV}, 12.5^{V}$ (4.1)	$33^{IV}, 67^{V}$ (4.7)
	$30^{IV}, 70^{V}$ (4.7)	$71^{IV}, 29^{V}$ (4.3)	$87.5^{IV}, 12.5^{V}$ (4.1)		$67^{IV}, 33^{V}$ (4.3)	$75^{IV}, 25^{V}$ (4.3)
5	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)
	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)
	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)	100^{V} (5.0)

Each line represents replicates; SC, solvent control; -, not tested
Each stage are separated with comma and denoted by superscripted Roman number
Stage is expressed in parenthesis

Table 4.11 Percent stage composition of sampled *Macrobrachium rosenbergii* larvae exposed to TBTO

Time	TBTO ($\mu g\ l^{-1}$)			
(day)	C	SC	0.6	1.2
1	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)
	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)
	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)
2	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)
	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)
	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)	100^{I} ($n = 5$)
3	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)
	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)
	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)
4	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)
	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)
	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)	100^{II} ($n = 5$)
5	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)
	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)
	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)

Table 4.11 cont.

Time	TBTO ($\mu g\ l^{-1}$)			
(day)	C	SC	0.6	1.2
6	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)
	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)
	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)	100^{III} ($n = 5$)
7	100^{IV} ($n = 5$)	100^{IV} ($n = 5$)	100^{IV} ($n = 5$)	100^{IV} ($n = 5$)
	100^{IV} ($n = 5$)	100^{IV} ($n = 5$)	100^{IV} ($n = 5$)	100^{IV} ($n = 5$)
	100^{IV} ($n = 5$)	100^{IV} ($n = 5$)	100^{IV} ($n = 5$)	100^{IV} ($n = 5$)
8	100^{V}($n = 5$)	100^{V}($n = 5$)	100^{V}($n = 5$)	100^{V}($n = 5$)
	100^{V}($n = 5$)	100^{V}($n = 5$)	100^{V}($n = 5$)	100^{V}($n = 5$)
	100^{V}($n = 5$)	100^{V}($n = 5$)	100^{V}($n = 5$)	100^{V}($n = 5$)
9	100^{VI} ($n = 10$)	100^{VI} ($n = 10$)	50^{V}, 50^{VI} ($n = 10$)	60^{V}, 33^{VI}, 7^{VII}($n = 15$)
	100^{VI} ($n = 10$)	100^{VI} ($n = 10$)	53^{V}, 40^{VI}, 7^{VII} ($n = 15$)	20^{IV}, 40^{V}, 40^{VI} ($n = 10$)
	100^{VI} ($n = 10$)	100^{VI} ($n = 10$)	50^{V}, 50^{VI} ($n = 10$)	10^{IV}, 60^{V}, 30^{VI} ($n = 10$)
10	100^{VI} ($n = 10$)	100^{VI} ($n = 10$)	47^{V}, 47^{VI}, 6^{VII} ($n = 15$)	50^{V}, 43^{VI}, 7^{VII} ($n = 14$)
	100^{VI} ($n = 10$)	100^{VI} ($n = 10$)	50^{V}, 40^{VI}, 10^{VII} ($n = 10$)	70^{V}, 20^{VI}, 10^{VII} ($n = 10$)
	100^{VI} ($n = 10$)	100^{VI} ($n = 10$)	40^{V}, 60^{VI} ($n = 10$)	80^{V}, 20^{VII} ($n = 10$)

Table 4.11 cont.

Time	TBTO ($\mu g\ l^{-1}$)			
(day)	C	SC	0.6	1.2
11	100^{VI} (n = 10)	100^{VI} (n = 10)	50^{V}, 50^{VI} (n = 10)	64^{V}, 36^{VI} (n = 14)
	100^{VI} (n = 10)	100^{VI} (n = 10)	60^{V}, 30^{VI}, 10^{VII} (n = 10)	10^{IV}, 40^{V}, 50^{VI} (n = 10)
	100^{VI} (n = 10)	100^{VI} (n = 10)	60^{IV}, 40^{V}, 10^{VI} (n = 10)	50^{V}, 40^{VI}, 10^{VII} (n = 10)
12	50^{VI}, 50^{VII} (n = 10)	20^{V}, 60^{VI}, 20^{VII} (n = 10)	20^{V}, 80^{VI} (n = 10)	50^{V}, 50^{VI} (n = 10)
	100^{VI} (n = 10)	50^{V}, 50^{VI} (n = 10)	40^{V}, 60^{VI} (n = 10)	50^{V}, 40^{VI}, 10^{VII} (n = 10)
		20^{V}, 10^{VI}, 60^{VII}, 10^{VIII} (n = 10)	50^{V}, 50^{VI} (n = 10)	80^{V}, 20^{VI} (n = 10)
13	20^{V}, 60^{VI}, 20^{VII} (n = 10)	20^{V}, 60^{VI}, 20^{VII} (n = 10)	30^{V}, 60^{VI}, 10^{VII} (n = 10)	40^{V}, 60^{VI} (n = 10)
	20^{V}, 80^{VI} (n = 10)	50^{V}, 50^{VI} (n = 10)	40^{V}, 60^{VI} (n = 10)	25^{V}, 37.5^{VI}, 37.5^{VII} (n = 8)
		10^{V}, 90^{VI} (n = 10)	80^{V}, 20^{VI} (n = 10)	70^{V}, 30^{VI} (n = 10)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		20^{V}, 80^{VI} (n = 10)	20^{V}, 80^{VI} (n = 10)	20^{V}, 80^{VI} (n = 5)
16	10^{V}, 20^{VI}, 40^{VII}, 30^{VIII} (n = 10)	20^{VI}, 30^{VII}, 50^{VIII} (n = 10)	20^{V}, 40^{VI}, 40^{VII} (n = 10)	60^{V}, 40^{VI} (n = 5)
	60^{VII}, 40^{VIII} (n = 10)	70^{VI}, 10^{VII}, 10^{VIII} (n = 10)	40^{V}, 20^{VI}, 40^{VII} (n = 10)	40^{V}, 20^{VI}, 40^{VII} (n = 5)
		20^{V}, 80^{VI} (n = 10)	20^{V}, 70^{VI}, 10^{VIII} (n = 10)	60^{VI}, 40^{VII} (n = 5)

Table 4.11 cont.

Time	TBTO ($\mu g\ l^{-1}$)			
(day)	C	SC	0.6	1.2
17	10^{V}, 20^{VI}, 20^{VII}, 50^{VIII} ($n = 10$) 10^{VI}, 50^{VII}, 40^{VIII} ($n = 10$)	30^{VI}, 20^{VII}, 50^{VIII} ($n = 10$) 10^{V}, 20^{VI}, 50^{VII}, 20^{VIII} ($n = 10$) 10^{V}, 90^{VI} ($n = 10$)	10^{V}, 50^{VI}, 40^{VII} ($n = 10$) 40^{V}, 40^{VI}, 20^{VII} ($n = 10$) 30^{V}, 60^{VI}, 10^{VII} ($n = 10$)	40^{V}, 40^{VI}, 20^{VII} ($n = 5$) 20^{V}, 40^{VI}, 40^{VII} ($n = 5$) 20^{V}, 60^{VI}, 20^{VII} ($n = 5$)
18	10^{V}, 10^{VI}, 20^{VII}, 50^{VIII}, 10^{IX} ($n = 10$) 20^{V}, 20^{VII}, 60^{VIII} ($n = 10$)	10^{V}, 40^{VI}, 10^{VII}, 30^{VIII}, 10^{IX} ($n = 10$) 11^{V}, 56^{VI}, 33^{VIII} ($n = 9$) 67^{VI}, 22^{VII}, 11^{VIII} ($n = 9$)	40^{VI}, 40^{VII}, 20^{VIII} ($n = 5$) 30^{V}, 40^{VI}, 30^{VII} ($n = 10$) 10^{V}, 80^{VI}, 10^{VII} ($n = 10$)	25^{V}, 75^{VI} ($n = 4$) 20^{V}, 40^{VI}, 40^{VII} ($n = 5$) 60^{VI}, 20^{VII}, 20^{VIII} ($n = 5$)
19	10^{V}, 30^{VII}, 50^{VIII}, 10^{IX} ($n = 10$) 10^{VI}, 30^{VII}, 60^{VIII} ($n = 10$)	25^{VII}, 62.5^{VIII}, 12.5^{IX} ($n = 8$) 67^{VI}, 22^{VII}, 11^{IX} ($n = 9$) 20^{V}, 70^{VI}, 10^{VII} ($n = 10$)	20^{VI}, 40^{VII}, 40^{VIII} ($n = 5$) 30^{V}, 40^{VI}, 60^{VII} ($n = 10$) 10^{V}, 80^{VI}, 10^{VIII} ($n = 10$)	40^{V}, 60^{VII} ($n = 5$) 20^{V}, 80^{VII} ($n = 5$)
20	20^{V}, 10^{VI}, 30^{VIII}, 40^{IX} ($n = 10$) 10^{VI}, 30^{VII}, 20^{VIII}, 40^{IX} ($n = 10$)	60^{VII}, 40^{VIII} ($n = 5$) 20^{VI}, 60^{VII}, 20^{IX} ($n = 5$) 30^{V}, 40^{VI}, 30^{VII} ($n = 10$)	60^{VII}, 40^{VIII} ($n = 5$) 40^{V}, 20^{VI}, 40^{VII} ($n = 5$) 40^{V}, 20^{VI}, 40^{VII} ($n = 5$)	20^{V}, 40^{VI}, 40^{VII} ($n = 5$) 20^{V}, 40^{VI}, 40^{VII} ($n = 5$)
21	10^{V}, 10^{VI}, 10^{VII}, 10^{VIII}, 50^{IX}, 10^{X} ($n = 10$) 10^{VI}, 30^{VII}, 40^{VIII}, 20^{IX} ($n = 10$)	20^{VI}, 20^{VII}, 20^{VIII}, 40^{IX} ($n = 5$) 20^{V}, 20^{VI}, 20^{VII}, 20^{VIII}, 20^{XI} ($n = 5$) 40^{VI}, 40^{VII}, 20^{VIII} ($n = 5$)	40^{VI}, 60^{VIII} ($n = 5$) 20^{V}, 80^{VII} ($n = 5$) 80^{VI}, 20^{VIII} ($n = 5$)	40^{VI}, 40^{VII}, 20^{VIII} ($n = 5$) 40^{VI}, 60^{VII} ($n = 5$)

Table 4.11 cont.

Time	TBTO ($\mu g\ l^{-1}$)			
(day)	C	SC	0.6	1.2
22	20^{VIII}, 60^{IX}, 20^{X} ($n = 5$) 20^{VIII}, 80^{IX} ($n = 5$)	20^{VI}, 60^{VII}, 20^{X} ($n = 5$) 40^{VII}, 20^{VIII}, 20^{IX}, 20^{X} ($n = 5$) 40^{VI}, 20^{VII}, 20^{VIII}, 20^{X} ($n = 5$)	40^{VI}, 60^{VIII} ($n = 5$) 20^{V}, 60^{VI}, 20^{VII} ($n = 5$) 80^{VI}, 20^{VII} ($n = 5$)	20^{VI}, 40^{VII}, 40^{VIII} ($n = 5$) 20^{VI}, 60^{VII}, 20^{VIII} ($n = 5$)
23	80^{VIII}, 20^{X} ($n = 5$) 100^{VIII} ($n = 5$)	20^{VI}, 60^{VII}, 20^{X} ($n = 5$) 40^{VII}, 20^{VIII}, 20^{IX}, 20^{X} ($n = 5$) 40^{VI}, 20^{VII}, 20^{VIII}, 20^{IX} ($n = 5$)	40^{VI}, 40^{VIII}, 20^{IX} ($n = 5$) 20^{V}, 40^{VI}, 40^{VII} ($n = 5$) 20^{V}, 60^{VI}, 20^{VIII} ($n = 5$)	14^{V}, 14^{VI}, 29^{VII}, 43^{VIII} ($n = 7$) 20^{VI}, 40^{VII}, 40^{VIII} ($n = 5$)
24	20^{VII}, 20^{VIII}, 60^{X} ($n = 5$) 20^{VIII}, 80^{X} ($n = 5$)	20^{VI}, 20^{VII}, 20^{VIII}, 40^{XI} ($n = 5$) 25^{VI}, 25^{VII}, 25^{VIII}, 25^{XI} ($n = 4$) 20^{V}, 40^{VI}, 40^{VII} ($n = 5$)	20^{VI}, 20^{VII}, 40^{VIII} ($n = 5$) 40^{VI}, 40^{VII}, 20^{VIII} ($n = 5$)	20^{VI}, 20^{VII}, 60^{VIII} ($n = 5$) 20^{VI}, 40^{VII}, 40^{VIII} ($n = 5$)
25	20^{VII}, 60^{X}, 20^{XI} ($n = 5$) 40^{VIII}, 40^{IX}, 20^{XI} ($n = 5$)	20^{VI}, 20^{VII}, 20^{VIII}, 20^{X}, 20^{XI} ($n = 5$) 60^{VII}, 20^{IX}, 20^{XI} ($n = 5$)	40^{VI}, 20^{VII}, 40^{VIII} ($n = 5$) 40^{VI}, 40^{VII}, 20^{VIII} ($n = 5$)	20^{VI}, 20^{VII}, 60^{VIII} ($n = 5$) 20^{VI}, 20^{VII}, 40^{VIII}, 20^{X} ($n = 5$)
26	20^{VIII}, 20^{IX}, 60^{X} ($n = 5$) 20^{VIII}, 60^{IX}, 20^{XI} ($n = 5$)	20^{V}, 20^{VII}, 60^{X} ($n = 5$) 60^{VII}, 40^{VIII} ($n = 5$)	40^{VI}, 20^{VII}, 40^{VIII} ($n = 5$) 40^{VI}, 20^{VII}, 40^{VIII} ($n = 5$)	20^{VI}, 20^{VII}, 60^{VIII} ($n = 5$) 25^{VII}, 50^{VIII}, 25^{IX} ($n = 4$)

Table 4.11 cont.

Time	TBTO ($\mu g\ l^{-1}$)			
(day)	C	SC	0.6	1.2
29	$50^{VIII}, 25^{X}, 25^{P}$ ($n = 4$)	$67^{VIII}, 33^{X}$ ($n = 3$)	$40^{VI}, 20^{VII}, 40^{VIII}$ ($n = 5$)	$50^{VII}, 50^{VIII}$ ($n = 4$)
	$25^{VIII}, 25^{IX}, 25^{X}, 25^{P}$ ($n = 4$)	$33^{VII}, 67^{X}$ ($n = 3$)	$40^{VI}, 60^{VIII}$ ($n = 5$)	$50^{VIII}, 50^{IX}$ ($n = 2$)
30	$67^{IX}, 33^{X}$ ($n = 3$)	$67^{VIII}, 33^{P}$ ($n = 3$)	$25^{VI}, 25^{VII}, 50^{VIII}$ ($n = 4$)	$33.3^{VII}, 33.3^{VIII}, 33.3^{X}$ ($n = 3$)
	$33^{VIII}, 67^{P}$ ($n = 3$)	$33.3^{VII}, 33.3^{X}, 33.3^{P}$ ($n = 3$)	$25^{VI}, 75^{VIII}$ ($n = 4$)	100^{VII} ($n = 3$)

C, control; SC, solvent control

Each stage are separated with comma and denoted by superscripted Roman number, superscripted P denoted early postlarval stage

Each line represents replicates

n = sampling numbers

Table 4.12 Larval developmental rate after exposed to TBTO

Time	TBTO ($\mu g\ l^{-1}$)			
(day)	C	SC	0.6	1.2
1	1.0±0.0 (1.0±0.0)	1.0±0.0 (1.0±0.0)	1.0±0.0 (1.0±0.0)	1.0±0.0 (1.0±0.0)
2	2.0±0.0 (2.0±0.0)	2.0±0.0 (2.0±0.0)	2.0±0.0 (2.0±0.0)	2.0±0.0 (2.0±0.0)
3	2.0±0.0 (2.0±0.0)	2.0±0.0 (2.0±0.0)	2.0±0.0 (2.0±0.0)	2.0±0.0 (2.0±0.0)
4	4.0±0.0 (3.0±0.0)	4.0±0.0 (3.0±0.0)	4.0±0.0 (3.0±0.0)	4.0±0.0 (3.0±0.0)
5	4.0±0.0 (3.0±0.0)	4.0±0.0 (3.0±0.0)	4.0±0.0 (3.0±0.0)	4.0±0.0 (3.0±0.0)
6	7.0±0.0 (4.0±0.0)	7.0±0.0 (4.0±0.0)	7.0±0.0 (4.0±0.0)	7.0±0.0 (4.0±0.0)
7	10.0±0.0 (5.0±0.0)	10.0±0.0 (5.0±0.0)	10.0±0.0 (5.0±0.0)	10.0±0.0 (5.0±0.0)
8	10.0±0.0 (5.0±0.0)	10.0±0.0 (5.0±0.0)	10.0±0.0 (5.0±0.0)	10.0±0.0 (5.0±0.0)
9	14.0±0.0 (6.0±0.0)	14.0±0.0 (6.0±0.0)	12.1±0.0 (5.5±0.0)	10.8±0.0 (5.2±0.0)
10	14.0±0.0 (6.0±0.0)	14.0±0.0 (6.0±0.0)	12.3±0.0 (5.6±0.0)	11.4±0.0 (5.4±0.0)
11	14.0±0.0 (6.0±0.0)	14.0±0.0 (6.0±0.0)	12.0±0.0 (5.5±0.0)	11.4±0.0 (5.4±0.0)
12	14.8±0.8 (6.3±0.3)	12.9±0.9 (5.7±0.3)	12.2±0.2 (5.6±0.1)	12.2±0.3 (5.6±0.1)
13	15.1±1.3 (6.4±0.4)	13.2±0.5 (5.8±0.1)	12.8±0.4 (5.7±0.1)	11.8±0.6 (5.5±0.2)
15	16.4±1.8 (6.8±0.5)	14.5±1.3 (6.2±0.4)	12.9±0.3 (5.7±0.1)	13.2±0.0 (5.8±0.0)
16	17.4±0.8 (7.1±0.3)	14.1±0.9 (6.0±0.3)	13.7±0.1 (5.9±0.3)	14.4±1.0 (6.1±0.3)

Table 4.12 cont.

Time	TBTO ($\mu g\ l^{-1}$)			
(day)	C	SC	0.6	1.2
17	17.6±0.4 (7.2±0.3)	17.0±0.7 (7.0±0.2)	13.1±0.1 (5.8±0.0)**	14.1±0.3 (6.0±0.1)**
18	18.2±0.0 (7.4±0.0)	15.4 ±0.1 (6.5±0.1)	13.8±0.1 (6.0±0.0)*	15.1±0.7 (6.4±0.2)*
19	18.5±0.0 (7.5±0.0)	15.8±0.0 (6.6±0.0)	14.0±0.3 (6.0±0 1)*	14.9±0.7 (6.3±0.2)
20	20.3±0.3 (8.1±0.1)	19.7±1.9 (7.9±0.6)	13.7±0.1 (6.0±0.1)*	14.4±0.0 (6.1±0.0)*
21	20.1±0.6 (8.0±0.2)	19.1±0.7 (7.7±0.2)	15.4±0.2 (6.5±0.1)*	16.1±0.3 (6.7±0.1)*
22	23.6±0.4 (8.9±0.1)	19.8±0.0 (7.9±0.0)	14.5±0.7 (6.2±0.2)**	17.3±0.3 (7.1±0.1)*
23	20.8±0.6 (8.2±0.2)	18.6±0.0 (7.5±0.0)	14.4±0.0 (6.1±0.0)**	17.3±0.3 (7.7±0.1)
24	25.3±1.1 (9.4±0.3)	21.6±1.1 (8.4±0.3)	16.7±0.3 (6.9±0.1)*	17.9±0.3 (7.3±0.1)*
25	25.1±1.3 (9.3±0.3)	21.6±0.4 (8.4±0.1)	16.7±0.3 (6.9±0.1)**	19.0±0.8 (7.7±0.2)*
26	25.1±0.5 (9.3±0.1)	20.2±2.0 (8.1±0.5)	17.0±0.0 (7.0±0.0)*	17.2±1.0 (7.1±0.5)*
29	26.5±5.5 (9.7±1.5)	23.5±4.5 (8.9±1.1)	17.3±0.3 (7.1±0.1)*	20.3±1.7 (8.1±0.5)
30	28.0±2.7 (10.1±0.8)	26.2±0.9 (9.6±0.3)	18.2±0.5 (7.4±0.1)*	20.9±0.9 (8.2±0.2)

C, control; SC, solvent control

Data represent mean age±S.D. in days as staged according to Uno and Kwon chin soo, 1969. Stage is expressed as mean±S.D. in parenthesis.

* $P < 0.05$

** $P < 0.01$