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Bioaccumulation and Elimination of Tributyltin and Triphenyltin in Oysters and Rock Shells in Taiwan

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ABSTRACT

Bioaccumulation and elimination of tributyltin (TBT) and triphenyltin (TPhT) in different culture mediums (A, control; B, 0.40 μg-TBT/L; C, 0.40 μgTPhT/L; and D, 0.20 μg-TBT/L + 0.20 (gTPhT/L) for 15, 30, 45 and 60 days in oysters (*Crassostrea gigas*) and for 59, 73 and 92 days in rock shells (*Thais clavigera*) have been carried out at Tungkang Marine Laboratory. The test animals were collected from the northern (Shiangsan: 24˚46'02" N, 120˚54'05" E) and southern (Chiku: 23˚03' 45" N, 120˚04'37" E) oyster mariculture areas on the west coast of Taiwan in August 2000. Results indicated that no hermaphroditic oyster was observed when cultured in different TBT and TPhT mediums for 60 days. For rock shells, after 92 days, only male (TBT, 2188 ± 21 ng/g) survived in medium B; while both females (TPhT, 2107 ± 30 ng/g) and males (TPhT, 2013 ± 17 ng/g) were found in medium C. No elimination of TBT and TPhT in imposex, female and male rock shells were observed while such elimination was found in both female and male oysters. High bioaccumulation rates of TBT in male (oyster, 10.7 ng/g/day; rock shells, 55.5 ng/g/day) and that of TPhT in female (oysters, 14.5 ng/g/day; rock shells, 27.1 ng/g/day) as well as high rates of TBT (20.0 ng/g/day) and TPhT (24.6 ng/g/day) in imposex rock shells were observed. Some experimental results on the effects of bioaccumulation and elimination of TBT and TPhT on sexual characteristics (male, female, imposex or hermaphroditic) of rock shells and oysters were discussed and evaluated.

Key words: tributyltin, triphenyltin, rock shells, oysters, sexual phenomena, hermaphroditic, imposex.

INTRODUCTION

Organotin compounds such as tributyltin (TBT) and triphenyltin (TPhT), widely used as biocidal agents (plant protection, antifouling paints for ships, boats and fishing nets), catalysts and stabilizers for polyvinyl chloride polymers, have become sources of marine pollution in coastal areas[1-5]. The compounds of TBT and TPhT can be degraded to dibutyltin (DBT), monobutyltin (MBT), diphenyltin (DPhT) and monophenyltin (MPhT), respectively, by solar radiation, bacterial biodegradation, and/or biological decomposition[6].

While it is difficult to estimate the consumption pattern of TBT and TPhT in Taiwan and how much TBT and TPhT is released into the coastal water, the Taiwan Agriculture Industry Association[7] indicated more than 150 tons of 45 % and 20 tons of 2% TPhT acetate were used for agricultural purposes in 1996. Ship building and ship repairing industries as well as passing vessels, have contributed unknown quantities of organotins into the Taiwan Strait. Therefore, it is not surprising to find high contents (dry weight) of TBT (as high as 2500 ng/g) in dumpsite sediment of Keelung Harbor dredged mud[8], and high concentrations of TBT (as high as 1510 ng/g) in oysters (*Crassostrea gigas*) from the Shiangsan Mariculture Area in the winter of 1997; and high concentrations of TPhT (as high as 590 ng/g) in the Lukang Area in the summer of 1996[9]. Extremely high values of TBT were observed in hermaphroditic oyster samples collected in the winter of 2000 compared with the low values in the female and male collected in the summer and autumn of 1999[10]. Rock shells (*Thais clavigera*) live alongside oysters in the same environment. Imposex, the imposition of male sex characteristics, notably a pseudopenis on female, was first observed in rock shells in Taiwan in January 1999. The higher the concentration of TBT in rock shells, the higher percentages of imposex was observed. Linear correlations of pseudopenis length versus TBT ($r = 0.7655$, $p < 0.001$), DBT ($r = 0.4253$, $p < 0.05$), MBT ($r = 0.5865$, $p < 0.01$) and TPhT ($r = -0.6160$, $p < 0.01$) were significant[11]. The purpose of this study was to explore the toxicity, bioaccumulation and elimination of TBT and TPhT in oysters and rock shells in the mariculture environments.

EXPERIMENTAL METHODS

Samples of oysters (*C. gigas*) and rock shells (*T. clavigera*) were collected from the northern (Shiangsan:

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24°46'02" N, 120°54'05" E) and southern (Chiku: 23°03'45" N, 120°04'37" E) oyster mariculture areas on the west coast of Taiwan in August 2000. Immediately after collection, the samples were transferred into Tungkang Marine Laboratory for the study on toxicity, bioaccumulation and elimination of TBT and TPhT. The bioaccumulation and elimination of TBT and TPhT in animals were studied in different culture mediums: A, control; B, 0.40 μg-TBT/L; C, 0.40 μg-TPhT/L; and D, 0.20 μg-TBT/L + 0.20 μg-TPhT/L. After each period (15, 30, 45, and 60 days for oysters; 59, 73 and 93 days for rock shells), individuals showing various sexual characteristics: male, female, hermaphroditic (both male and female) oysters and imposex rock shells were identified and separated[10-11].

The pooled flesh samples (8.00 ~ 10.00 g) were treated with tropolone-benzene solution and then analyzed for the species of organotins [such as TBT, DBT (dibutyltin), MBT (monobutyltin), TPhT, DPhT (diphenyltin) and MPhT (monophenyltin)] by the GC/FPD method[9]. To determine the species of organotins in oysters, all organotin species (purities ranging from 95 to 98%, obtained from Aldrich-Chemie, Steinhein, Germany) and tetrabutyltin (as an internal standard) as well as the Standard Reference Material (SRM, NIES. No. 11; fish (Leteolabrax japonicus, Cuvier tissue) were used for QA/QC checks. The average recoveries (n = 3) for TBT, DBT, MBT, TPhT, DPhT and MPhT were 92.4 ± 6.9%, 93.5 ± 8.0%, 85.6 ± 4.0%, 40.4 ± 5.0%, 97.3 ± 10.8% and 75.2 ± 7.0%, respectively. The detection limits analyzed by the GC/FPD method (dry weight) were 9.1 ng/g for TBT, 13.4 ng/g for DBT, 8.1 ng/g for MBT, 15.7 ng/g for TPhT, 7.2 ng/g for DPhT and 8.7 ng/g for MPhT with this method.

RESULTS AND DISCUSSION

I. *Bioaccumulation and Elimination of TBT and TPhT in Oysters*

Oysters *(C. gigas)* collected from Shiangsan were cultured in four different culture mediums (A, control; B, 0.40 μg-TBT/L; C, 0.40 μg-TPhT/L; and D, 0.20 μg-TBT/L with 0.20 μg-TPhT/L) for 15, 30, 45 and 60 days. No hermaphroditic oysters were observed when cultured at different concentrations of TBT and TPhT mediums up to 60 days. The concentrations of TBT and TPhT in both female and male oysters increased with culture time. However, female oysters contained much higher TBT and TPhT than male oysters. For instance, after 60 days in mediums B and D, the concentrations of TBT increased from 124.9 ± 9.4 to 686.9 ± 23.9 ng/g and 98.4 ± 7.2 to

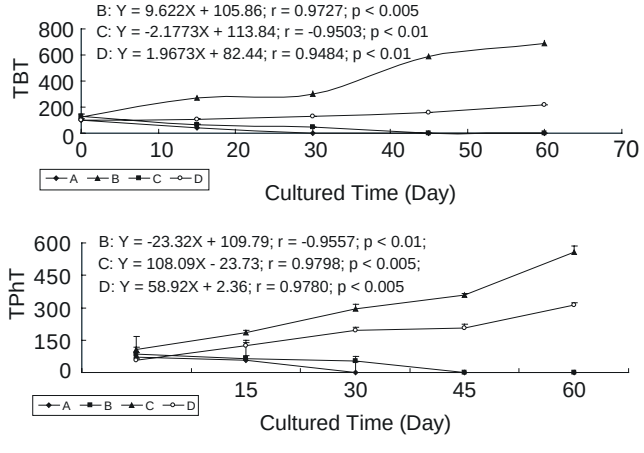

Figure 1. Organotin contents (ng/g, dry weight) in female oysters (*C. gigas*) observed in different cultural media (A: control; B: 0.4 μg-TBT/L; C: 0.4 μg-TPhT/L; and D: 0.2 μg-TBT/L + 0.2 μg-TPhT/L).

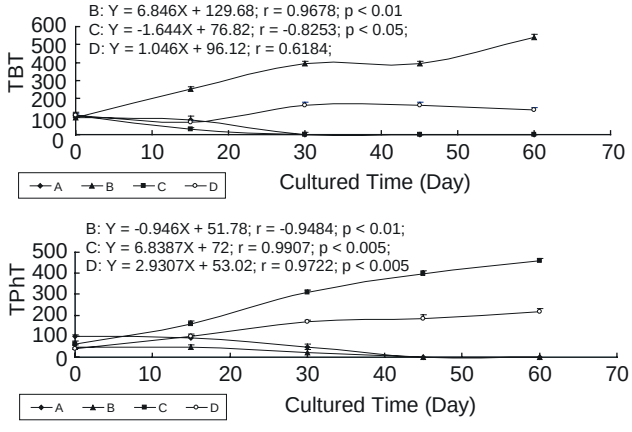

Figure 2. Organotin contents (ng/g, dry weight) in male oysters *(C. gigas)* observed in different cultural media (A: control; B: 0.4 μg-TBT/L; C: 0.4 μg-TPhT/L; and D: 0.2 μg-TBT/L + 0.2 μg-TPhT/L).

Table 1. Bioaccumulation and elimination rates (ng/g/day) of organotin in oysters (*Crassostrea gigas*) and rock shells (*Thais clavigera*) cultured in different media [A, control; B, 0.4 μg-TBT/L; C, 0.4 μg-TPhT/L; D, (0.2 μg-TBT + 0.2 μg-TPhT)/L; ♂, Male; ♀, Female; ♀ ♂, Imposex; OY, Oysters; RS, Rock shells; SS, Shiangsan; CK, Chiku].

	Med	Rate	♂, OY	♂, RS	♀,OY	♀,RS	♀ ♂,RS
SS/TBT	B	Vo	10.5	15.7	10.3	20.1	20.0
		V_1	-1.8	10.4	-0.6	13.6	7.6
		V_2	10.7	55.5	9.8		
	D	Vo	-3.8	9.7	0.2	8.9	10.6
		V_1	7.7	4.5	1.8	6.0	7.2
		V_2	-1.4		4.3		
SS/TPhT	C	Vo	6.1	23.0	8.6	23.3	24.6
		V_1	10.4	7.7	3.8	8.4	6.0
		V_2	4.5	21.2	14.5	27.1	
	D	Vo	4.1	11.0	4.7	12.9	14.5
		V_1	0.8	6.0	-0.1	10.3	16.7
		V_2	2.3		7.9		
CK/TBT	B	Vo		18.6		16.6	20.6
		V_1		6.9		22.1	5.0
	D	Vo		10.5		10.5	9.6
		V_1		15.9		7.3	12.2
		V_2		9.9			
CK/TPhT	C	Vo		24.6		22.1	26.4
		V_1		8.3		14.5	2.3
		V_2		30.9		36.7	
	D	Vo		14.8		15.8	16.4
		V_1		6.5		19.1	8.1
		V_2		21.9		7.8	

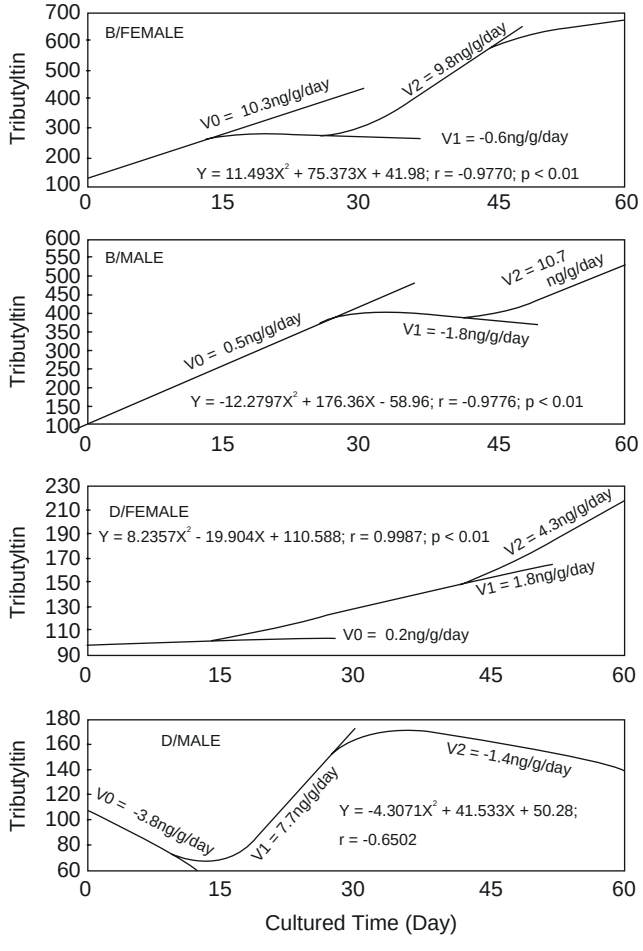

Figure 3. Bioaccumulation and elimination rates (ng/g/day) of butyltins (dry weight) in oysters (*C. gigas*) cultured in different media (B, 0.4 μg-TBT/L; D, 0.2 μg-TBT/L + 0.2 μg-TPhT/L).

218.4 ± 16.8 ng/g in females (Figure 1); and from 94.7 ± 12.1 to 537.9 ± 18.2 ng/g and 107.4 ± 7.1 to 138.9 ± 11.5 ng/g in males (Figure 2), respectively. After 60 days in mediums C and D, the concentrations of TPhT increased from 105.1 ± 11.4 to 559.1 ± 26.7 ng/g and 58.3 ± 6.9 to 312.8 ± 11.9 ng/g in females; and from 64.1 ± 12.9 to 459.1 ± 12.4 and 38.7 ± 6.6 to 215.1 ± 17.2 ng/g in males, respectively. Interestingly, positive correlations were found between the culture time and organotin concentrations in oysters cultured in different mediums (Table 2).

For oysters cultured in medium B, the initial bioaccumulation rates (Vo) of TBT in females (10.3 ng/g/day) and males (10.5 ng/g/day) were similar (Figure 3). After initial bioaccumulation, a higher elimination rate (V_1) of TBT in males (-1.8 ng/g/day) was observed than that in females (-0.6 ng/g/day). For oysters cultured in medium D, low bioaccumulation rates (Vo, 0.2 ng/g/day; V_1, 1.8 ng/g/day; and V_2, 4.3 ng/g/day) of TBT were found in female oysters; and high elimination rates (Vo, -3.8 ng/g/day; V_2, -1.4 ng/g/day) were observed in males. Similar to the TBT pattern, when oysters were cultured in mediums C and D, higher initial (Vo) and final bioaccumulation rates (V_2) of TPhT were generally found in female oysters (C: Vo, 8.6

Table 2. Correlations between the culture time and organotin (TBT and TPhT) concentrations in oysters cultured in different media.

oysters	Correlation culture time vs TBT conc.		Correlation culture time vs TPhT conc.	
	Medium B	Medium D	Medium C	Medium D
Male	r = 0.9678	p < 0.01	None	r = 0.9907
	p < 0.005		r = 0.9722	p < 0.005
Female	r = 0.9798	p < 0.005	r = 0.9484	p < 0.005
	r = 0.9798	p < 0.005	r = 0.9780	p < 0.005

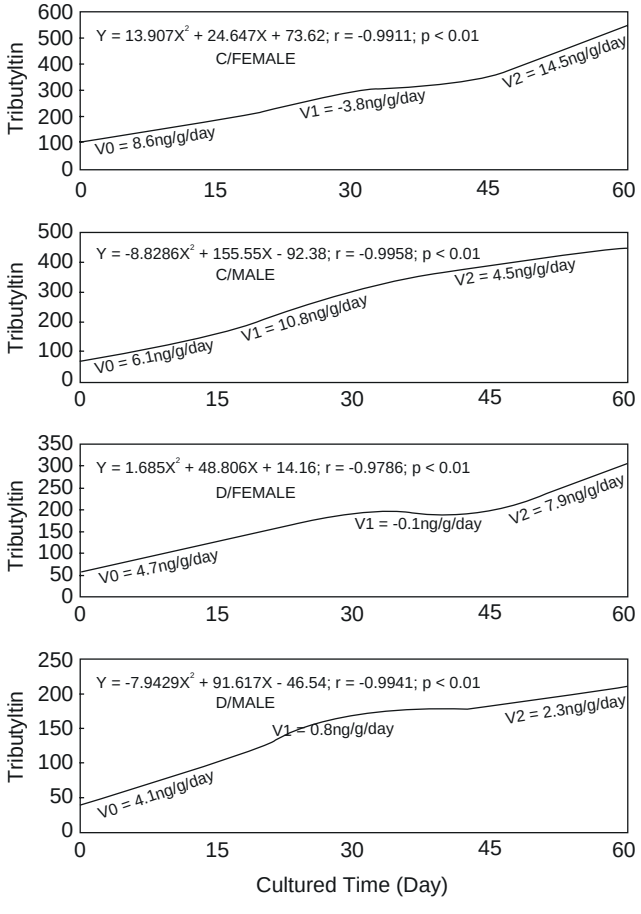

Figure 4. Bioaccumulation and elimination rates (ng/g/day) of phenyltims (dry weight) in oysters (*C. gigas*) cultured in different media (B, 0.4 μg-TBT/L; D, 0.2 μg-TBT/L + 0.2 μg-TPhT/L).

ng/g/day; V_2, 14.5 ng/g/day; D: Vo, 4.7 ng/g/day; V_2, 7.9 ng/g/day) compared with those rates (C: Vo, 6.1 ng/g/day; V_2, 7.9 ng/g/day; D: Vo, 4.1 ng/g/day; V_2, 2.3 ng/g/day) in males (Figure 4). This might be the reason why the concentrations of TBT and TPhT in females (Figure 1) were generally higher than those in males (Figure. 2) when cultured in mediums B, C, and D for 60 days.

II. *Bioaccumulation and Elimibation of TBT and TPhT in Rock Shells*

Rock shells *(T. clavigera)*, collected from the northern (Shiangsan) and southern (Chiku: 23˚03'45" N, 120˚04'37" E) oyster mariculture areas, were cultured in different

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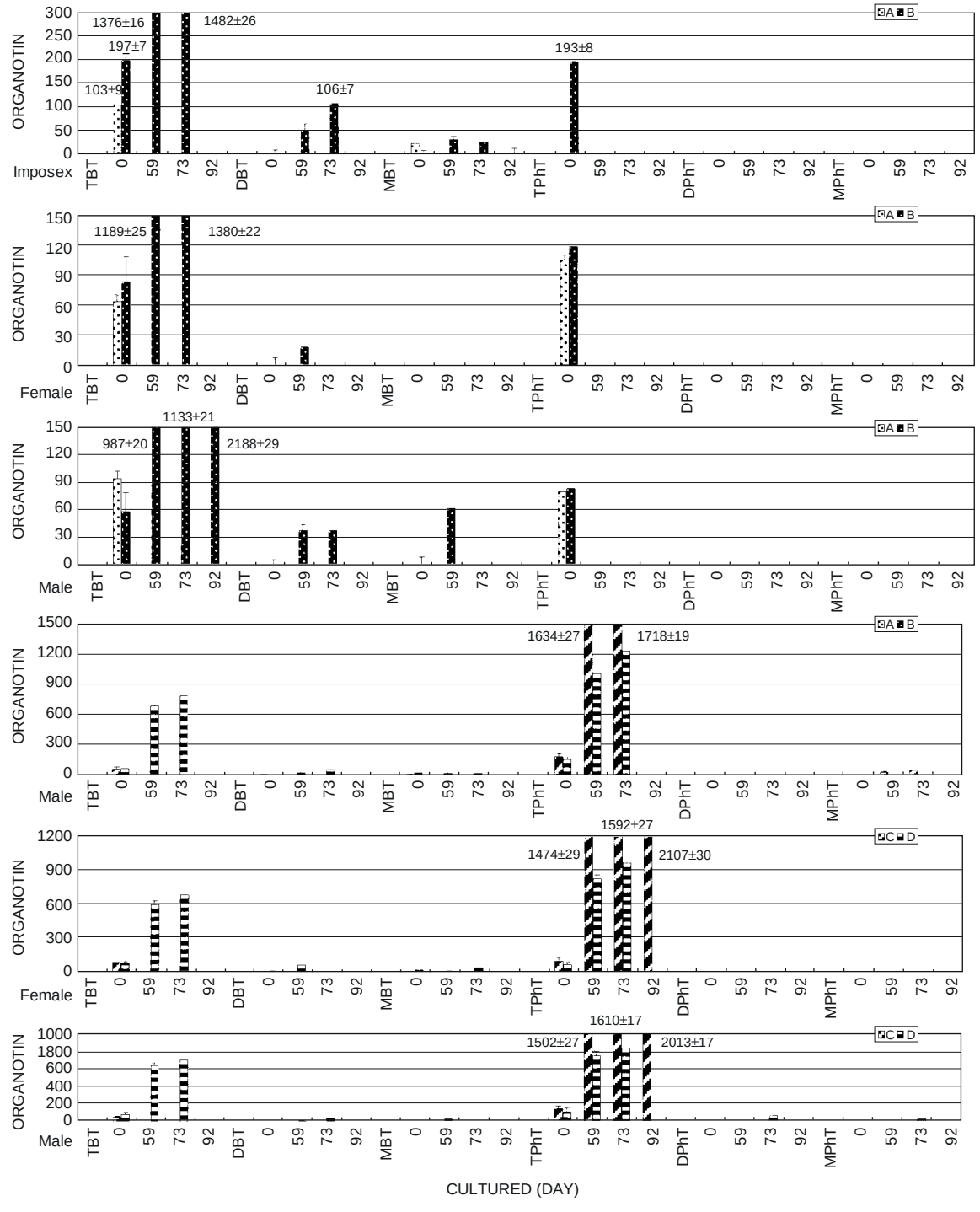

Figure 5. Bioaccumulation of organotin (ng/g, dry weight) in rock shells *(Thais clavigera)* cultured in different mediums (A: control; B: 0.4 μg-TBT/L; C: 0.4 μg-TPhT/L; D: 0.2 μg-TBT/L + 0.2 μg-TPhT/L; n: ♀ ♂, 47; ♀, 30; ♂, 43; Cultured animals collected from Shiangsan mariculture area in August 2000)

mediums (A, control; B, 0.40 μg-TBT/L; C, 0.40 μg-TPhT/L; and D, 0.20 μg-TBT/L + 0.20 μg-TPhT/L) at Tung-Kang Marine Laboratory. After culturing for 59, 73, and 92 days, samples were taken for studying the sexual characteristics (male, female or imposex) and analyzed for organotin species (TBT, DBT, MBT, TPhT, DPhT and MPhT). In principle, the higher initial concentrations of TBT and TPhT in rock shells, the higher bioaccumulation of TBT and TPhT were observed. The results shown in Figures. 5 and 6 indicated that imposex in rock shells gave higher TBT and TPhT concentrations than in female and male rock shells. For example, when cultured in mediums B for 73 days (Figures. 5 and 6), the TBT values in imposex animals (Shiangsan, 1482 ± 26 ng/g; Chiku, 1368 ± 19 ng/g) were higher than those in females (Shiangsan, 1380 ± 22 ng/g; Chiku, 1358 ± 18 ng/g) and males

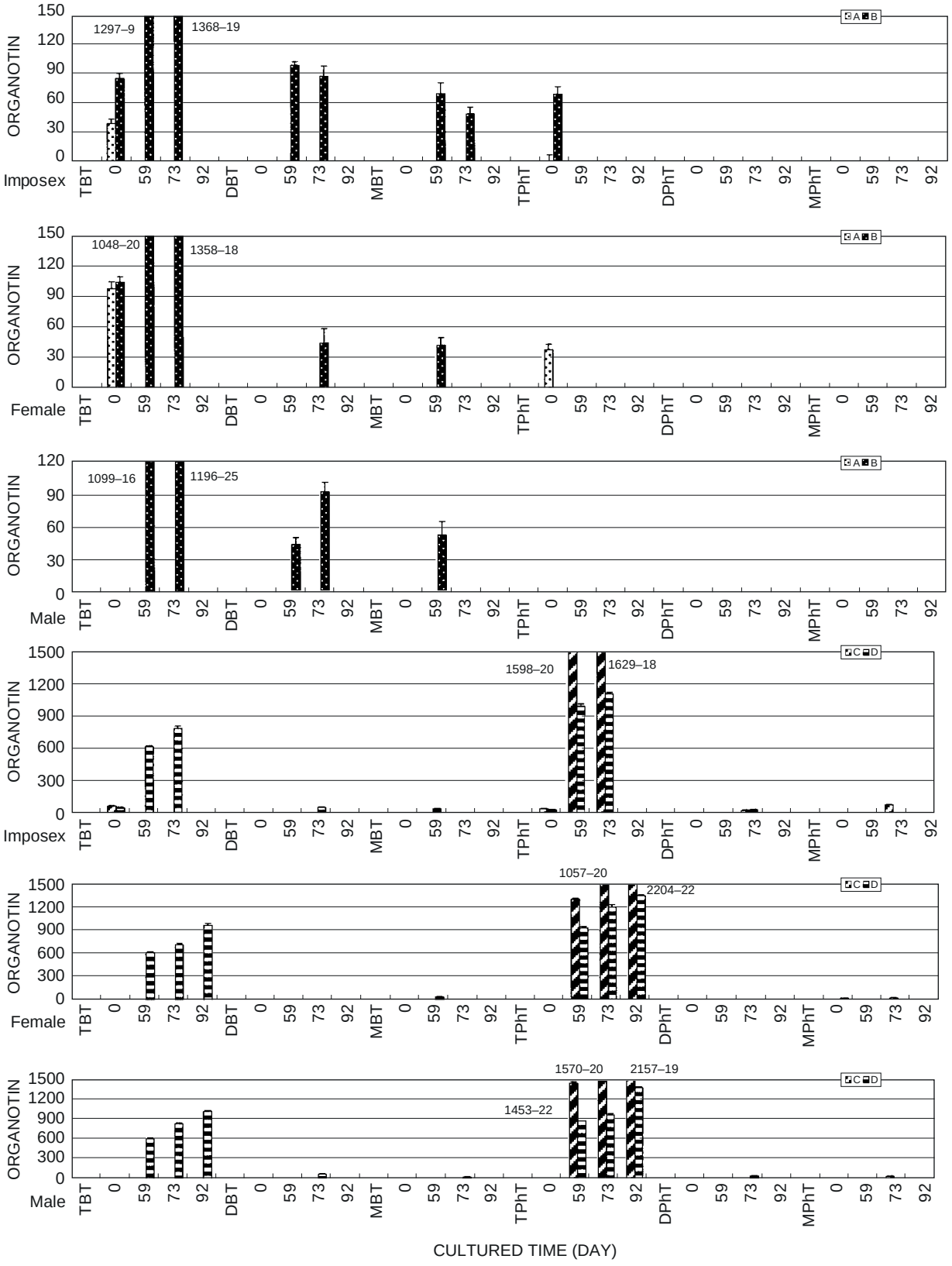

Figure 6. Bioaccumulation of organotin (ng/g, dry weight) in rock shells *(Thais clavigera)* cultured in different mediums (A: control; B: 0.4 μg-TBT/L; C: 0.4 μg-TPhT/L; D: 0.2 μg-TBT/L + 0.2 μg-TPhT/L; n: ♀ ♂, 40; ♀, 41; ♂, 39; Cultured animals collected from the Chiku mariculture area in August 2000).

(Shiangsan, 1133 ± 21 ng/g; Chiku, 1196 ± 25 ng/g). The concentrations of TPhT in imposex animals in medium C (Shiangsan, 1718 ± 19 ng/g; Chiku, 1629 ± 18 ng/g) were higher than those in males (Shiangsan, 1610 ± 17 ng/g;

Chiku, 1570 ± 20 ng/g) and females (Shiangsan, 1592 ± 27 ng/g; Chiku, 1507 ± 22 ng/g). During the cultural period, the concentrations of DBT, MBT, DPhT and MPhT were low. According to previous studies[10-11], the major factor causing imposex in rock shells was TBT. After 92 days, only male rock shells (TBT, 2188 ± 21 ng/g) survived in medium B; while both male (TPhT, 2013 ± 17 ng/g) and female (TPhT, 2107 ± 30 ng/g) rock shells were found in medium C.

No elimination of TBT and TPhT in imposex, female or male rock shells was observed while elimination was obtained in female and male oysters when culturing rock shells together with oysters in mediums B, C and D (Table 1). For the Shiangsan Mariculture Area, rock shells showed high bioaccumulation rates of TBT in male (10.7 ng/g/day for oyster and 55.5 ng/g/day for rock shells) and TPhT in female (14.5 ng/g/day for oysters and 27.1 ng/g/day for rock shells) as well as high rates of TBT (20.0 ng/g/day) and TPhT(24.6 ng/g/day) in imposex of rock shells.

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REFERENCES

1. Meinema, H. A., Van Dam-Meerbeek, T. G. & Vonk, W. J. 1986. Evaluation of impact of organotin compounds on the aquatic environment. Final Rept. Of TNO, Utrecht to EEC. Contract No. 85/B/600/11/007/11/N.
2. Alzieu, C. 1989. Monitoring and assessment of butyltins in Atlantic coastal water. Mar. Pollut. Bull. 20: 22-26.
3. Alzieu, C. 1991. Environmental problems caused by TBT in France: assessment, regulation, prospects. Mar. Environ. Res. 32: 7-17.
4. Salazer, M. H. & Salazer, S. M. 1991. Assessing site-specific effects of TBT contamination with mussel growth rates. Mar. Environ. Res. 32: 131-150.
5. Iwata, H., Tanabe, S., Mizuno, T. & Tatsukawa, R. 1995. High accumulation of toxic butyltins in marine mammals from Japanese coastal waters. Environ. Sci. & Technol. 29: 2959-2962.
6. Maguire, R. J., Carey, J. H. and Hale, E. J. 1983 Degradation of the tributyltin species in water. J. Agric. Food Chem. 31: 1060-1065.
7. TAIA 1997 Domestic manufactures production and sale of pesticides in 1996. Taiwan Agriculture Industry Association.
8. Hung, T. C. and Liu, B. P. 1998 Determination of tributyltin in sediments from the Machu and Taiwan coastal areas. Acta Oceanogr. Taiwanica 37: 105-112.
9. Hung, T. C., Lee, T. Y. and Liao, T. F. 1998 Determination of butyltins and phenyltins in oysters and fishes from Taiwan coastal waters. Environ. Pollut. 102: 197-203.
10. Hung, T. C., Hsu, W. K., Meng, P. J. and Chuang, A. 2001 Organotins and imposex in the rock shells, *Thais clavigera*, from the Taiwan oyster mariculture area. Environ. Pollut. 112: 145-152.
11. Hung, T. C., Hsu, W. K., Meng, P. J. and Chuang, A. 2000 Species of organotins in imposex of rock shells and hermaphroditic oysters from the western coast of Taiwan. Bull. Inst. Chem., Academia Sinica 47: 1-12.