BUTYLTIN RESIDUES IN SEDIMENTS FROM THE EASTERN GULF OF THAILAND

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ABSTRACT

Concentrations of monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT) compounds were analyzed in surface sediment samples collected from twelve stations along the Eastern Gulf of Thailand. The total butyltin (∑BTs) concentrations ranged between <1 and 109.7 ng g^{-1} (dry weight). The overall concentration ranges found in the sediments were from <1 to 79.81 ng g^{-1} for MBT, from <1 to 9.5 ng g^{-1} for DBT, and from <1 to 29.8 ng g^{-1} for TBT, on a dry weight basis. MBT generally prevailed in most of the samples, suggesting the occurrence of old inputs of butyltin compounds in the area. Butyltin compounds were also measured in *Nassarius* sp., where ∑BTs concentrations were found to range between <1 and 238 ng g^{-1} (wet weight). The level of butyltin compounds in gastropod tissue samples tended to be related to that of the sediments and percentage of imposex incidence.

Keywords: TBT, organotins, imposex, gastropods, Gulf of Thailand

INTRODUCTION

Tributyltin (TBT) has been known as the favored biocide for use in antifouling paints since the 1970s. Due to its effective performance against all fouling species, TBT was extensively used worldwide and widely contaminated the marine environment (water, sediment and organisms), including Thailand's coastal waters, (Kan-atireklap *et al*., 1997; Tanabe *et al*., 2000; Sudaryanto *et al*., 2004; Harino *et al*., 2006; Wattayakorn, 2008). In addition, the deleterious effects of TBT released by antifouling paints also caused severe damage to non-target organisms, such as shell deformation in oyster (Alzieu, 1991) and imposex in gastropods (Gibbs and Bryan, 1986). Imposex is an irreversible symptom which has been reported by many researchers (Bryan *et al*., 1986; Bech *et al*., 2002). The severity of imposex depended on the level of contamination of TBT (Bryan *et al*., 1986). TBT could accumulated and persist in sediment over long periods, hence becoming a source of recontamination to the environment by sediment resuspension (Stewart and de Mora, 1990; Dowson *et al*., 1994; Page *et al*., 1996; Smith, 1996; Reitsema *et al*., 2003; Wattayakorn, 2008). Therefore, adverse effects on marine organisms can still occur even after the total banning of TBT (Stewart and de Mora, 1990; Wattayakorn, 2008; Langston *et al*., 2009).

In Thailand, butyltin (BT) contamination in coastal sediments were first reported by Kan-atireklap *et al*. (1997) with TBT, DBT and MBT concentrations ranged from 4 to 4,500, 2 to 1,900 and 7 to 410 ng g^{-1} (dry weight), respectively. The average percentage of TBT, DBT and MBT were 52%, 21% and 27%, respectively. The concentration of TBT was higher in far-seas vessel harbours than fishing boat piers/ports and mariculture sites, with the ratios of TBT in BTs were 72%, 50-56% and 46%, respectively. The re-surveys of contaminated sediments in the Gulf of Thailand were conducted in the year 2000

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(Lommetta, 2001) and 2004 (Harino *et al*., 2006) in which the amounts of BTs found tended to have declined since the first study (Table 1). Lommeta (2001) conducted a study along the Eastern Gulf of Thailand and reported TBT contamination in the sediments in the range between 2-115 ng g^{-1} dry weight. The average proportion of TBT/∑BT was 40%, while the level of DBT and MBT were 2-190 ng g^{-1} and 7-91 ng g^{-1} dry weight, respectively. Harino *et al.* (2006) studied BTs contamination in the inner Gulf of Thailand and reported the level range from 2 to 1,246 ng g^{-1}, from 1 to 368 ng g^{-1} and from 1 to 292 ng g^{-1} dry weight for TBT, DBT and MBT, respectively. The average ratio of TBT/∑BT was 44% higher than DBT (24%) and MBT (32%). Butyltin contamination in marine organisms has also been of concern in Thailand's coastal waters. The amount of butyltin compounds in various marine organisms was studied, such as in green mussels (Kan-Atireklap *et al*., 1997; Lommetta, 2001; Harino *et al*., 2006), cockles and oysters (Lommetta, 2001), marine mammals: dugongs, whales and dolphin (Harino *et al*., 2007a; 2007b; 2008) and gastropods (Bech, 2002; Bech *et al*. 2002).

Butyltin contamination in tissue samples together with imposex occurrence were studied in Southern Thailand. Bech (2002) studied the butyltin concentration in *Chicoreus capucinus* which located about 100 m far from the Yatch Haven Marina, Phuket Island found that the trend of butyltin in the tissue sample was higher after the marina's construction was completed. Bech *et al*. (2002) studied the concentration of butyltin in the tissue of *Thais distinguenda* after transplant into a TBT-contaminated area (Laem Tukkae, Phuket Island). The amount of BTs during the period of transplant ranged between 4.8 – 40.0 and 4.8 – 39.1 ng Sn g^{-1} dry weight in big and small-sized samples, respectively. However, TBT tissue burden was not correlated with the time after transplant or the incidence of imposex.

Although TBT usage has been banned in Thailand and concentration levels of BTs in the Thai waters tended to have declined, TBT could accumulate and persist in sediment over long periods and even extremely low amount of BTs can affect organisms. Hence, further study on distribution of butyltin compounds are still needed in order to assess the potential impact of BTs on the coastal environment of Thailand.

MATERIALS AND METHODS

Twelve surface sediment samples were collected from coastal areas along the Eastern Gulf of Thailand (Fig. 1) during May–Aug, 2011

Table 1. BTs contamination in Thailand coastal sediments

Year	Location	MBT	DBT	TBT	References
		ng g^{-1} (dry weight)			
1995	Thailand coastal area	7 - 410	2 - 1,900	4 - 4,500	Kan-atireklap *et al*. (1997)
2000	Eastern Gulf of Thailand	7 - 91	2 - 190	2 - 115	Lommetta (2001)
2004	Inner Gulf of Thailand	1 -292	1 - 368	2 - 1246	Harino *et al*. (2006)

to analyze for BTs contamination (TBT, DBT and MBT). These areas include harbours with intense maritime activities, fishery ports, industrial areas with discharging effluents, coastal mariculture areas and tourism activities (Table 2). Some marine gastropods were also collected to study the level of butyltin compounds. *Nassarius* sp. was chosen in this study because these species showed the highest imposex occurrence at the same study sites (Praditsup and Chaitanawisuti, 2011) as the present study. Hence, *Nassarius* sp. was collected from stations 5, 9, 10 and 12 to quantify for BTs concentrations. After collection, both sediment and tissue samples were kept frozen until analysis. Extraction was done by the method of liquid-liquid extraction and followed by gas chromatography with a flame photometric detector as described by Harino (2003) with a slight modification; i.e. using n-propyl magnesium chloride in the propylation step instead of n-propyl magnesium bromide. Briefly, the sample was extracted twice with 25ml acetone for 10 min. After removal the supernatant, organic layer was extracted with 25% NaCl solution and 0.1% tropolone-benzene

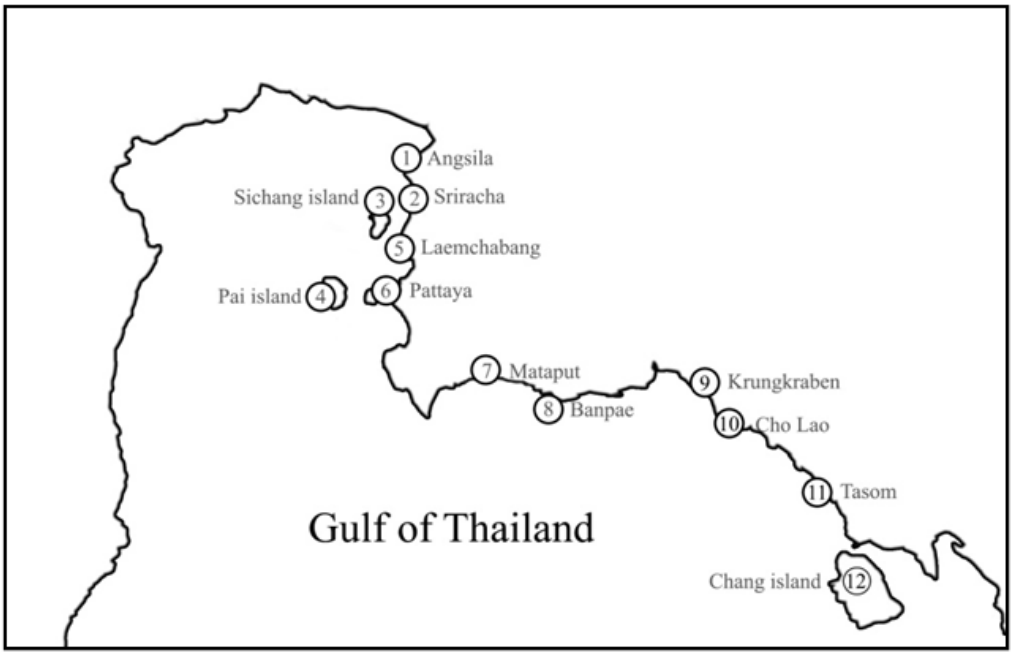

Figure 1. Map of sampling locations in the Eastern Gulf of Thailand**.**

Table 2. Sampling location**,** the Eastern Gulf of Thailand

Station	Location	Coastal Activities
1	Angsila, Chonburi Province	coastal mariculture area
2	Sriracha, Chonburi Province	large cargo offshore port
3	Sichang Island, Chonburi Province	island opposite to Siracha station
4	Pai Island, Chonburi Province	tourism site
5	Laemchabang, Chonburi Province	deep sea port
6	Pattaya, Chonburi Province	tourism site
7	Mataput, Rayong Province	industrial estate
8	Banpae, Rayong Province	tourism sites, small fishing area
9	Krungkraben, Chantaburi Province	coastal mariculture area
10	Chao Lao, Chantaburi Province	small fishing boat pier
11	Tasom, Trad Province	small fishing area
12	Chang island, Trad Province	tourism site, small fishing boat pier

for 10 min. The separated fraction was extracted again with 0.1% tropolone-benzene solution. For the sediment sample, the co-extracted inorganic sulphur was removed by mixing the organic layer with 3.3% tetrabutylammonium hydrogen sulphate and 16% sodium sulphite. The concentrated extract which already removed water was filled up with benzene and propylated by added 3ml of n-propylmagnesium chloride in diethyl ether (ca.2M) and shaken gently for 10 min at 40ºc in a water-bath. The mixture was added with 10 ml of 1 N H_2SO_4 and 40 ml of water. After that, the aqueous was extracted twice by 20 ml of hexane/ benzene (9/1). The water in combined organic layer was removed by anhydrous Na_2SO_4 and concentrated by rotary evaporator and the volume was made-up to 5 ml by hexane. The sample was cleaned by Florisil Sep-Pak cartridge, after that concentrated by rotary evaporator and 0.1ml of internal standard (TeBT, 2 mg/L) was added, finally filled up with hexane. Recoveries of MBT, DBT and TBT in the sediments were 81.6 ± 6.9%, 81.8 ± 17.0% and 88.4 ± 3.1%, respectively. For the tissue samples, the recoveries were 86.6% for MBT, 58.8 ± 12.7% for DBT and 42.3 ± 7.2% for TBT.

The imposex occurrence (%) was also checked in *Nassarius* sp., by which the percentage of imposex is the number of females with a pseudopenis present x 100 divided by the total number of females in the sample. The relative penis length index (RPLI) for each female was obtained from the equation: (mean length of female penis) / (mean length of male penis) x 100.

RESULTS

All study sites were detected to be contaminated by butyltin compounds. The total butyltin (∑BTs) concentrations were found in the range between <1 to 109.7 ng g^{-1} (dry weight). The overall concentration ranges found in the sediments were <1 – 79.8 ng g^{-1} for MBT, <1 – 9.5 ng g^{-1} for DBT, and <1 – 29.8 ng g^{-1} for TBT, on a dry weight basis. Comparing the average concentration of ∑BTs among the study sites indicated that high contamination areas were station 5 (Laemchabang) (32.8 ng g^{-1} dry weight) and station 2 (Siracha) (28.7 ng g^{-1} dry weight) (Fig. 2a), a deep sea port and a large cargo offshore port. Butyltin compositions at station 5 were MBT>TBT>DBT, with concentrations of MBT, DBT and TBT as 24.4, <1 and 8.4 ng g^{-1} (dry weight), respectively. At station 2, butyltin compositions showed the same trend as station 5, with the amount of MBT, DBT and TBT as 15.0, 3.2 and 10.5 ng g^{-1} (dry weight), respectively. Station 3 (an island opposite to station 2) and station 7 (the area near the industrial estate) showed less contamination levels than stations 5 and 2, with an average total butlytin concentration of 10.5 and 2.5 ng g^{-1} dry weight. Stations 1, 4, 6, 8-12 which are either coastal mariculture areas, small fishing boat piers or tourism sites, were found to have varied total butyltin contamination in the range of 2.1-13.7 ng g^{-1} (dry weight). The concentration of ∑BTs at station 1, 4, 6, 8-12 were 6.6, 9.6, 9.8, 13.7, 9.5, 5.8, 2.1 and 5.3 ng g^{-1} (dry weight), respectively.

In this study, MBT generally prevailed in most of the samples (Fig. 2). The average proportion of MBT/∑BTs was 67.6% while the proportions of DBT/∑BTs and TBT/∑BTs were 2.5% and 29.9%, respectively. At the highest contamination sites, station 2 and 5, the percentage proportion of MBT, DBT and TBT in ∑BTs ranged from 52-74%, <1-11% and 26-37%, respectively. Although the concentration of the others stations were not as high, some stations such as station 6, 9 and 10 showed higher proportions of TBT/∑BTs (64.4–97.7 %), while the percentage of DBT and MBT were <1 – 10 % and 2.1 – 35.4 %.

Butyltin compounds were analysed in whole tissue samples of some marine gastropods, *Nassarius* sp. (Nassaridae) which were sampled from stations 5, 9, 10, and 12. The total butyltin concentrations in *Nassarius* sp. were detected to range between <1 to 238 ng g^{-1} (wet weight). MBT, DBT and TBT were found between <1 to 157 ng g^{-1}, <1 to 48 ng g^{-1} and <1 to 33 ng g^{-1} on the wet weight basis, respectively. Comparing tissue contamination among collecting sites, the highest tissue burden was found at station 5 (Table 3) with the proportion of MBT/∑BT dominant (65.9%) while the percentage of DBT and TBT were 20.1 and 14.0. The tissue contamination also high in *N. pullus* at station 12, where the concentration of MBT, DBT and TBT were 80.3, 23.8 and 10.9 ng g^{-1} (wet weight). The proportion of MBT/∑BT also dominated (69.8%). However, at station 10 it was

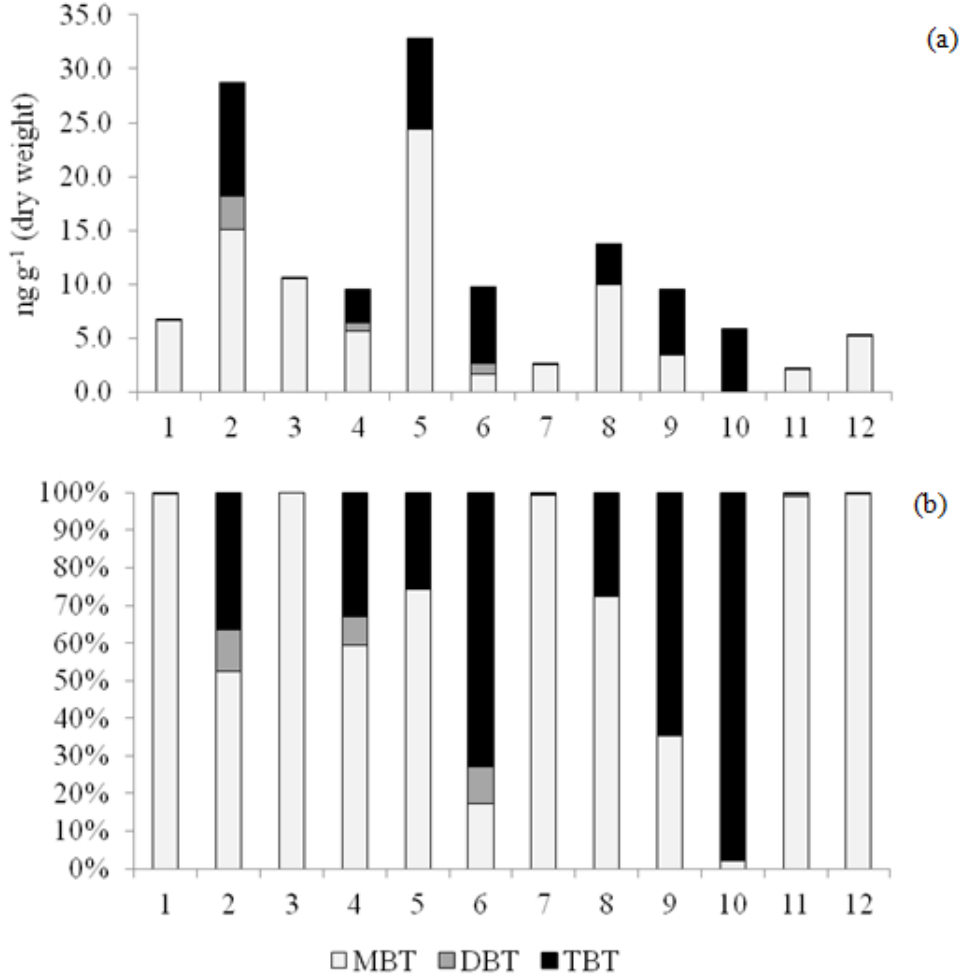

Figure. 2. Average concentration (a) and proportion (b) of butyltin compounds in surface sediments along the Eastern Gulf of Thailand.

Table 3. BTs contamination in gastropod tissue samples and imposex occurrence

Gastropod species	Station	MBT	DBT	TBT	% imposex	% RPLI
Nassarius livescens						
	5	156.9	47.9	33.3	95 (n = 20)	34.8
	10	<1	<1	6.6	29.2 (n = 24)	69.5
Nassarius pullus						
	9	<1	<1	<1	0 (n = 18)	0
	10	<1	3.1	4.9	70 (n = 10)	19.2
	12	80.3	23.8	10.9	-	

Table 4. The percentage proportion of MBT, DBT and TBT in ∑BTs of sediment samples

Location	Year	MBT	DBT	TBT	References
Thailand coastal area	1995	27.1	21.3	51.5	Kan-atireklap *et al*. (1997)
Eastern Gulf of Thailand	2000	37	23	40	Lommetta (2001)
Inner Gulf of Thailand	2004	32.4	24.0	43.6	Harino *et al*. (2006)
Eastern Gulf of Thailand	2011	67.6	2.5	29.9	This study

found that the proportion of TBT/∑BT was higher than DBT/∑BT and MBT/∑BT. For the study of imposex, the highest occurrence was found at station 5 (95%) in *N. livescens*. At station 10, 70 and 29.2 percent were recorded in *N. pullus* and *N. livescen*s, respectively. However, no imposex was found at station 9. RPLI was also measured in *Nassarius* sp. with the highest was found in *N. livescen*s at station 10 (69.5%) while the same species at station 5 was 34.8% (Table 3).

DISCUSSION

In this study, butyltin compounds were detected in all sediment samples collected along the Eastern Gulf of Thailand, although levels of contamination and butyltin compositions varied among sampling points. High BTs contamination was found at stations 5 and 2 which are intensive shipping activity areas. Several studies reported that high levels of activity of large sea vessels and/or shipyard areas were associated with high BTs contamination more than small fishing boat piers and mariculture areas (Kan-atireklap *et al*., 1997; Sudaryanto *et al*., 2004; Harino *et al*., 2008). Harino *et al*. (2006) and Wattayakorn (2008) suggested that the deposited TBT-contaminated paint-flakes were the cause of high TBT contamination in sediment in the dockyard areas. Moreover, Stang *et al*. (1992) reported that sediment with TBT-contained paint-chips will be degraded slowly due to adsorption of TBT onto the sediment.

The average proportion of TBT/∑BTs in sediments was found to have declined in comparison to those reported earlier (Table 4), with MBT generally prevailing in most of the samples (Fig. 2b). The ratio of TBT/∑BTs in the high contamination areas (station 2 and 5) was also reduced. Many researchers suggested that the ratio of TBT in ∑BTs could indicate the situation of TBT released in the environment; the lower the proportion of TBT/∑BTs, the lower the release of TBT (Ko *et al*., 1995; Tanabe *et al*., 2000; Sudaryanto *et al*., 2004; Harino *et al*., 2006; Arai and Harino, 2009). Hence, this study suggests old inputs of butyltin compounds in some studied areas. Some areas such as station 6, 9 and 10 showed a high proportion of TBT/∑BTs in the surface sediments, although the levels of ∑BTs were low (Fig. 2), indicating recent inputs of BTs in these areas.

Butyltin contamination in the gastropod tissues varied widely among stations. The highest BTs contamination in tissue of *N. livescens* was found at station 5 where the BTs contamination in sediments was also high. In addition, the percentage proportion of MBT in *N. livescens* (65.9% at station 5) and *N. pullus* (69.8% at station 12) were more dominant than DBT and TBT. On the other hand, the proportion of TBT was higher at station 10 for both *N. livescens* (almost 100%) and *N. pullus* (61.3%). These were in similar trends as the MBT, DBT and TBT proportions found in surface sediments at the same stations, which indicated that these scavenger gastropods can directly uptake BTs by ingesting the contaminated sediments as suggested by Berto *et al.* (2007).

Imposex is the unusual sexual characteristic induced on females by TBT as has been proved by many studies (Bryan *et al*., 1986; Bech *et al*., 2002; Laranjeiro *et al*., 2010). The degree of imposex depended on the exposure to TBT concentration (Bryan *et al*., 1986; Bech, 2002). The level of RPLI was also reported to be related to the degree of TBT exposure (Bech, 2002; Bech *et al*., 2002; Laranjeiro, *et al*., 2010). The percentage occurrence of imposex in this study was found to be highest at station 5 where the BTs contamination in the sediment and tissue were also high (Fig. 2, Table 3).

The results of this study showed that there is still evidence of TBT contamination in sediments from the Eastern Gulf of Thailand and, therefore, further studies to investigate the release of BTs from sediments and the ability of organisms to accumulate TBT are highly recommended.

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