

# Chapter 6

## Current Status of Organotin Contamination and ImPOSEX in Neogastropods Along Coastal Marine Environments of Southeast Asia and China

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**Abstract** Organotin compounds (OTs), in particular tributyltin and triphenyltin, have been contaminating various coastal marine environments around the world since their first application back in the 1920s. These compounds have been proven to adversely affect a wide range of marine organisms from microalgae to marine mammals, and they have a great potential of bioaccumulation via the food web. Some OTs such as triphenyltin can even be biomagnified through marine food chains. In Southeast Asia, OT contamination has been a widespread problem because most countries or regions do not implement local regulations to restrict the use of OT-based antifouling paints on seagoing vessels and fish farming facilities (e.g., open-sea cages), although some of them are members of the International Maritime Organization, which has enforced a global ban of such paints since September 2008. Contamination by OTs was the most severe in coastal waters where intensive shipping or mariculture activities could be found. To rectify the problem and safeguard the marine ecosystem and human health, long-term monitoring of OT contamination and enforcement of more stringent regulations on controlling the use and release of these pollutants in Southeast Asian countries are urgently needed.

**Keywords** Biomonitoring • Antifouling • Tributyltin • Triphenyltin • Ecological risk • Tissue burden • Endocrine disrupter • Marine ecosystem • South China • Hong Kong

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## 6.1 Overview

Organotin compounds (OTs), a group of organometallic compounds with a tin (Sn) atom covalently bonded to organic substituents, have been contaminating coastal marine environments worldwide ever since their first application as pesticides on high-value crops back in 1925 (Thompson et al. 1985; Hamlin and Guillette 2010). Among all OTs, the tri-substituted compounds, such as tributyltin (TBT) and triphenyltin (TPT), are found to have the greatest toxicological activity and, at the same time, the highest commercial value as effectual biocides (Fent 1996; Bao et al. 2011; Yi et al. 2012, 2014a, b). These compounds are then widely used in agricultural practices and textile industries as pesticides, fungicides, bactericides, and insecticides (Hoch 2001; Krupp et al. 2011; Lee and Chen 2011), as well as in antifouling systems on ship hulls and submerged fish farming facilities (Fent 1998). In Southeast Asia, TPT acetate and TPT chloride are even used as molluscicides in the shrimp farming industry (Gräslund and Bengtsson 2001). To date, phenyltin compounds are still widely used in various industrial products, and their contamination is widespread in China because monophenyltin has been frequently detected in sediment samples from coastal waters of South China (Zhang et al. 2013). The global production of OTs in the industry had been increasing annually to couple with their growing demand, from <5000 tonnes in 1955 to 35,000 tonnes in 1986, and a further increase to 50,000 tonnes in 1992 (WHO 1980; Huang et al. 1993; Hoch 2001). Although data of global OT production are lacking and the demand is thought to have been reduced in recent years, the production and usage of these compounds still remain prominent. For instance, China consumes at least 7500 tonnes of OTs every year (Jiang 2001; Cao et al. 2009), and produces at least 200 tonnes of TPT annually (Hu et al. 2009). Between 1994 and 1996, Japan also exported up to 140 tonnes of TPT for antifouling paints, although their internal use of TPT had ceased after 1990 (WHO 1999).

OTs, in particular butyltin and phenyltin compounds, are highly persistent in the marine environment, extremely toxic, and highly bio-accumulative. Therefore, these two groups of compounds are considered as being the highest ecological concern (Huang et al. 1993; Fent 1996). Goldberg (1986) expressed that “TBT was perhaps the most toxic substance ever deliberately introduced to the marine environment by mankind.” The adverse effects of OTs have called for management actions against these compounds on international stages. The Marine Environment Protection Committee (MEPC) of the International Maritime Organization (IMO) adopted a resolution in 1990 that recommended the elimination of the use of TBT-containing antifouling paints on non-aluminium-hulled vessels of less than 25 m in total length. In 1999, another resolution was adopted to call for a global ban of these paints. The legally binding convention, called the International Convention on the Control of Harmful Anti-fouling Systems on Ships (AFS Convention), was adopted in the IMO assembly in November 2001 (IMO 2008). The convention was then open for signature, and finally on 17 September 2007, Panama signed the convention as the 26th country ratifying the convention, and in total representing

38 % of the total tonnage of the world's fleet. The convention was finally entered into force on 17 September 2008 after a 1-year grace period (Sonak et al. 2009). Under the AFS Convention, all vessels cannot apply or re-apply OTs in their antifouling systems. All ship hulls should not bear OTs, and they should have a coating to cover the external parts of hulls to prevent such compounds from leaching into the marine environment (Cheung et al. 2010). Up until 31 August 2014, 68 countries or areas have ratified the AFS Convention, accounting for more than 83 % of the world's total tonnage (IMO 2014). Among the Southeast Asian countries or areas, only China, Malaysia, Singapore, and Macau (as associate member) have ratified the convention. Hong Kong has not yet signed the convention and Taiwan is not a member state of IMO. Thailand, the Philippines, East Timor, Indonesia, Brunei, Cambodia, Laos, and Myanmar have not ratified the convention either, and they all have very limited local restrictions of the use of OT-based antifouling systems.

Taking Hong Kong as an example, although Hong Kong is a member state of IMO it has not ratified the AFS Convention at the moment (IMO 2014). The only regulation regarding the use of OTs as antifouling paints was a partial ban on TBT-based antifouling paints on small vessels (i.e., <25 m in length) and open-sea cages, which was enacted in 1992 (Ko et al. 1995; ACE 1996). At the same time, TBT was deregistered from the list of pesticides under the Pesticide Ordinance Cap. 133, and the use of TBT was only allowed to those with a special permit (EPD 2008). However, there were still 11 registered boatyard users of TBT in 1995 (Ko et al. 1995), possibly continuously contaminating the marine environment of Hong Kong.

Taiwan did not sign the AFS Convention because it is not a member state of IMO (IMO 2014). However, there are some regulations concerning OTs locally. A prohibition of the use of pesticides containing TPT acetate was proposed by the Council of Agriculture in Taiwan in 1997. Triphenyltin compounds were then completely banned in agricultural practices in 1999. Tributyltin compounds were prohibited in the antifouling systems on small boats (<25 m in length) in 2003 (Meng et al. 2009). Other than these, no other regulations regarding OTs in water, sediment, flora, and fauna have been implemented in Taiwan so far (Hung et al. 2001).

On the other hand, as China is one of the countries ratifying the AFS Convention, it has the responsibility to implement national regulations to prohibit the use of OTs in antifouling systems concerning seagoing vessels and fish farming facilities (e.g., open-sea cages). However, such restrictions have not been implemented in any parts of China (Jiang et al. 2001; Cao et al. 2009). Together with the uncontrolled inland freshwater inputs of OTs (Lau et al. 2012), the contaminations have already caused widespread pollution problems in China, and the measured concentrations of OTs were far higher than international standards (Jiang 2001). Thus, academics have been calling for various actions to control the use and release of these pollutants into the marine environments of China for more than 10 years. These proposed actions include a continuous large-scale monitoring of OTs, an implementation of appropriate regulations to control the use of OT-containing antifouling

paints, an inclusion of OTs into water quality guidelines, an establishment of risk assessment procedures for these compounds, and an enhancement of the monitoring and educational programmes associated with the control of the use and release of these pollutants (Jiang 2001; Jin 2008; Cao et al. 2009; Gao et al. 2013).

Other Southeast Asian countries at present have no specific regulations on the use and release of OTs, including Malaysia (Tong et al. 1996) and Singapore (Tan 1997; Basheer et al. 2002). No relevant information can be found in the Philippines, East Timor, Indonesia, Brunei, Cambodia, Laos, and Myanmar. However, there is growing evidence showing that coastal marine waters of the southern Asian region have been extensively contaminated by OTs, posing health risks to the marine ecosystems and humans in this region.

## 6.2 Current Status of Organotin Contamination

As most Southeast Asian countries or regions do not have comprehensive regulations on the control of the use and release of OTs, contamination by OTs is commonly detected in water, sediment, and biota throughout this region (Tables 6.1, 6.2, and 6.3). Most documented studies have been concentrated on the distribution of TBT and other butyltin compounds in the water column, sediments, and biota. Although there is a growing concern about the contamination by TPT and other phenyltin compounds as well as their adverse impacts to marine organisms (Lee et al. 2005; Xie et al. 2010; Ho and Leung 2014b), the total number of relevant studies on phenyltin compounds is limited and scanty. Thus, historical comparisons of OT contamination would be limited to butyltin compounds.

### 6.2.1 Seawater

Table 6.1 summarises the concentrations of OTs measured in seawater samples collected in coastal environments of Southeast Asia and China during the past 27 years. In Hong Kong, a general decreasing trend of TBT was recorded during the past 20 years. Lau (1991) first measured the concentration of TBT in seawater, which reached  $1000 \text{ ng l}^{-1}$ . In sediment pore water, TBT concentration was detected up to  $610 \text{ ng l}^{-1}$  (Cheung et al. 2003). For sewage and stormwater discharge, the maximum concentration of butyltins detected was  $12.2 \text{ ng l}^{-1}$ , and TBT was only measured at  $1.1 \text{ ng l}^{-1}$  at maximum (Kueh and Lam 2008). However, the levels of TPT and other phenyltin compounds in seawater were only uncovered by a recent unpublished study, which revealed that the seawater was severely contaminated by TPT in different coastal areas of Hong Kong, in particular, at typhoon shelters (Ho et al. 2016). The total OT concentration ranged from 20.5 to  $41.9 \text{ ng l}^{-1}$ , at which TPT was the dominating compound among OTs, accounting for 45–63% of total OTs (Ho et al. 2016). The concentrations of

**Table 6.1** Concentrations of various organotin compounds in seawater in various locations of Southeast Asia and China: studies conducted between 1988 and 2014 of butyltins including mono-, di-, and tri-butyltin (TBT) and phenyltins encompassing mono-, di- and tri-phenyltin; total organotins are a sum of these six compounds

Country/ area	Location	Concentration	Year of study	References
Hong Kong	Various locations	TBT: <90–1000 ng l <sup>-1</sup>	1988–1989	Lau (1991)
	Victoria Harbour	TBT: n.d.–2740 ng l <sup>-1</sup>	1989	Chiu et al. (1991)
	Yam O, Tsing Yi	TBT: 120–610 ng l <sup>-1</sup>	NA	Cheung et al. (2003)
	Various water control zones	Butyltins: <2–5 ng l <sup>-1</sup>	2004	Kueh and Lam (2008)
	Sewage and river discharge	Butyltins: 0.0–12.2 ng l <sup>-1</sup>	2004	Kueh and Lam (2008)
	Various locations	Total organotins: 20.5–41.9 ng l <sup>-1</sup>	2013–2014	Ho et al. (2016)
China	Pearl River Delta	TBT: 21–39 ng l <sup>-1</sup>	1996	Chau (2005)
	Coastal cities of Northern China	Butyltins: 224.2 ng l <sup>-1</sup>	NA	Jiang (2001)
	Various locations along the coastline	Butyltins: 18.5–1273 ng Sn l <sup>-1</sup>	1998–1999	Jiang et al. (2001)
	Shantou	Butyltins: 338.76 ng l <sup>-1</sup>	2001–2002	Huang et al. (2005)
	Various locations along the eastern and southern coastline	Butyltins: 224.2 ng Sn l <sup>-1</sup>	2002	Zhou et al. (2002)
	Pearl River Delta	TBT: 21.7–38.5 ng l <sup>-1</sup>	NA	Fu et al. (2003)
	Bohai Bay	TBT: 0.0–14.7 ng l <sup>-1</sup>	2002	Gao et al. (2004)
	Huiyang	Butyltins: 3290.2 ng l <sup>-1</sup>	2002	Huang et al. (2005)
	Xiamen	Butyltins: 373.0 ng l <sup>-1</sup>	2002	Huang et al. (2005)
	Xiamen	TBT: 2.2–160 ng Sn l <sup>-1</sup>	2006	Wang et al. (2008)
	Shekou Harbour, Shenzhen	Butyltins: 191 ng l <sup>-1</sup> (TBT up to 152 ng l <sup>-1</sup> )	2007	Deng et al. (2008)
Taiwan	Kaohsiung	Butyltins: 90 ng l <sup>-1</sup>	1997–1998	Ou and Dong (1998) and Dong et al. (2004)
	Southern waters	Butyltins: 23.1–96.0 ng Sn l <sup>-1</sup> (TBT was below detection limit)	2002	Liu et al. (2002)
Malaysia	Various locations	TBT: <1.39–115 ng Sn l <sup>-1</sup>	1991–1992	Tong et al. (1996)
	Strait of Malacca	Butyltins: <0.24–5.1 ng Sn l <sup>-1</sup>	1996	Hashimoto et al. (1998)
Bay of Bengal	Various locations	Butyltins: <0.12–0.2 ng Sn l <sup>-1</sup>	1996	Hashimoto et al. (1998)

NA data not available, n.d. not determined

**Table 6.2** Concentrations of organotins in sediment in various locations of Southeast Asia and China from studies conducted during 1988–2014 of butyltins including mono-, di-, and tri-butyltin and phenyltins encompassing mono-, di-, and tri-phenyltin (dw, dry weight); total organotins are a sum of these compounds

Country/area	Location	Concentration	Year of study	References
China	Pearl River Delta	1.7–379.7 ng g <sup>-1</sup> dw	1997–1999	Zhang et al. (2003)
	Bohai Bay	Butyltins: 1.32–2.16 ng g <sup>-1</sup> dw	2011	An et al. (2013)
		Phenyltins: 5.14–7.21 ng g <sup>-1</sup> dw		
	Xiamen	n.d.–26 µg Sn kg <sup>-1</sup> dw	2006	Wang et al. (2008)
	Shantou	Butyltins: 5.1–35.9 ng g <sup>-1</sup> dw	2002	Huang et al. (2005)
Huiyang	Butyltins: 37.6–106 ng g <sup>-1</sup> dw	2002	Huang et al. (2005)	
Hong Kong	Various locations	n.d.–1160 ng g <sup>-1a</sup>	1988–1989	Lau (1991)
	Various locations	2.4–2837 ng g <sup>-1</sup> dw as TBT	NA	EPD (1998)
	Yam O	2–560 ng g <sup>-1</sup> dw	NA	Cheung et al. (2003)
	Tsing Yi	3.2–100 ng g <sup>-1</sup> dw	NA	Cheung et al. (2003)
	Victoria Harbour	129,486 ng g <sup>-1</sup> dw as TBT	1994	Ko et al. (1995)
		53,000 ng g <sup>-1</sup> dw as Sn		
	Aberdeen	44,709 ng g <sup>-1</sup> dw as TBT	1994	Ko et al. (1995)
		18,300 ng g <sup>-1</sup> dw as Sn		
Various locations	TBT: 1.5 (east of Hong Kong Island)–1163 (Tsing Yi) ng g <sup>-1</sup> dw as Sn	1998	Ma et al. (1998)	
Hebe Haven	MBT: 6.5 ng g <sup>-1</sup> dw as Sn	1998	Ma et al. (1998)	
	DBT: 37.5 ng g <sup>-1</sup> dw as Sn			
	TBT: 39.0 ng g <sup>-1</sup> dw as Sn			
Taiwan	Kaohsiung	57 ng g <sup>-1</sup> dw	1997–1998	Ou and Dong (1998) and Dong et al. (2004)
Malaysia		Butyltins: 5.9–1266 µg kg <sup>-1</sup> dw Phenyltins: 0.45–184 µg kg <sup>-1</sup> dw	2006	Harino et al. (2009)
		Butyltins: 14–1400 ng g <sup>-1</sup> dw	1997–1998	Sudaryanto et al. (2004)
Thailand		MBT: 7–410 ng g <sup>-1</sup> dw DBT: 2–1900 ng g <sup>-1</sup> dw TBT: 4–4500 ng g <sup>-1</sup> dw	1995	Kan-Atireklap et al. (1997)
		Butyltins: 3.3–1907 µg kg <sup>-1</sup> dw Phenyltins: 0.9–44.1 µg kg <sup>-1</sup> dw	2004	Harino et al. (2006)
		Butyltins: 1.55–43.6 µg kg <sup>-1</sup> dw	2002	Midorikawa et al. (2004)
		Phenyltins: 2.4–9.8 µg kg <sup>-1</sup> dw		

NA data not available

<sup>a</sup>The original literature did not mention whether the measured value was based on wet weight or dry weight

OTs were generally higher in summer than those in winter, probably because of the increased rainfall, surface runoff, and sewage discharge during summer.

Being the largest developing country in Southeast Asia and with wide applications of OTs, China has been reported to have heavy OT contamination in its seawater. Jiang et al. (2001) first extensively quantified the concentrations of butyltins (i.e., summation of MBT, DBT, and TBT) in various water bodies along the coastline of China and detected these compounds at concentrations up to 1273 ng Sn l<sup>-1</sup>, in which TBT was the dominant compound, ranging from below detection limit (0.5 ng Sn l<sup>-1</sup>) to 977 ng Sn l<sup>-1</sup>. Many important coastal cities in Southeast China were threatened by OTs. Xiamen, for instance, was shown to have concentrations of OTs in seawater ranging from 62.9 to 1128.1 ng l<sup>-1</sup> (total butyltins) and from 2.2 to 160 ng Sn l<sup>-1</sup> (TBT), respectively, in two separate studies (Huang et al. 2005; Wang et al. 2008). The southern part of China, being an area with rapid economic growth, is often regarded as the “world’s factory.” Because of the intensive marine and estuarine traffic associated with busy marine trades and fishing activities, coastal areas of South China generally receive greater contamination of OTs than other parts of China. For example, in Shekou Harbour of Shenzhen, the average concentration of total butyltins in seawater was 191 ng l<sup>-1</sup>, and TBT remained the dominant compound among butyltins at concentrations up to 152 ng l<sup>-1</sup>, reflecting the continuous input of TBT into the system (Deng et al. 2008). Although the average concentration of total butyltins in South China was comparable to those measured in northern parts of China (Jiang 2001), the highest concentration of TBT in southern waters was much higher than its residual standards of the U.S.A. and Canada by 8- and 80-fold, respectively (Deng et al. 2008). The results indicated the severity of OT contamination in the South China region. Water samples collected from the Pearl River Delta also showed severe contamination by TBT at concentrations ranging from 21.7 to 38.5 ng l<sup>-1</sup> (Fu et al. 2003), which were much higher than the standards given by the U.K. (2 ng l<sup>-1</sup>; Cleary 1992) and the U.S.A. (10 ng l<sup>-1</sup>; Federal Register 1989).

In Taiwan, Tang and Wang (2009) discovered a negative relationship between seawater TBT concentrations and the distance between a sampling point and an adjacent fishing port or harbour. Meng et al. (2009) summarised OTs concentrations detected in coastal waters of Taiwan and showed that the highest concentration of TBT, at 480 ng l<sup>-1</sup>, was found in a commercial harbour of Kaohsiung (Jang 2004). In coral reef areas, the concentrations of TBT were comparatively low, ranging from not detected to 17 ng l<sup>-1</sup> (Lee 2002). Phenyltin compounds were not detected in all samples.

In other parts of Southeast Asia, contaminations of OTs in seawater were poorly studied. Harino et al. (2008) summarised two studies of OT contamination in seawater from Malaysia. One of these studies revealed TBT at concentrations ranging from <1.39 to 115 ng Sn l<sup>-1</sup>, and the highest concentrations were observed at Selangor within the Strait of Malacca (Tong et al. 1996). In Singapore, TBT concentrations in seawater ranged from 0.43 to 3.2 µg l<sup>-1</sup>, and high concentrations

**Table 6.3** Concentrations of organotins in biota tissues collected from various locations in Southeast Asia and China from studies conducted during 1990–2014 of butyltins (dw) including mono-, di-, and tri-butyltin and phenyltins encompassing mono-, di-, and tri-phenyltin; total organotins are these six compounds

Taxa	Species	Location	Concentration	Year of study	Reference	
Gastropod	Rock shell, <i>Reishia clavigera</i>	Shantou, China	Butyltins: 47.40 ng g <sup>-1</sup> ww	2001	Huang et al. (2005)	
		Xiamen, China	Butyltins: 24.9–124.3 ng g <sup>-1</sup> ww	2001–2002	Huang et al. (2005)	
		Huiyang, China	Butyltins: 44.55 ng g <sup>-1</sup> ww	2002	Huang et al. (2005)	
		Pearl River Delta	TBT: <18.8 ng g <sup>-1</sup> ww	2003	Huang et al. (2005)	
		Hong Kong	Butyltins: <0.05–914.7 ng Sn g <sup>-1</sup> dw	2004	Leung et al. (2006)	
		Hong Kong	Butyltins: 19.1–177.0 ng Sn g <sup>-1</sup> dw	2005–2006	Qiu et al. (2011)	
		Shenzhen	Butyltins: 12.2–122.4 ng Sn g <sup>-1</sup> dw	2006	Chan et al. (2008)	
		Daya Bay and Dapeng Bay of Shenzhen	Butyltins: 15.8–70.8 µg kg <sup>-1</sup> dw Phenyltins: 5047.3–23,420.5 µg kg <sup>-1</sup> dw	2013	Ho and Leung (2014b)	
		Rock shell, <i>Thais</i> sp.	Sarawak, East Malaysia	MBT: 87.5–173 µg kg <sup>-1</sup> ww	NA	Mohamat-Yusuff et al. (2014)
				DBT: 18–269 µg kg <sup>-1</sup> ww		
				TBT: 1–33 µg kg <sup>-1</sup> ww		
				MPT: up to 154 µg kg <sup>-1</sup> ww		
DPT: up to 538 µg kg <sup>-1</sup> ww						
TPT: up to 25 µg kg <sup>-1</sup> ww						
Veined rapa whelk, <i>Rapana venosa</i>	Bohai Bay	Butyltins: 14.1–28.81 ng g <sup>-1</sup> dw	2011	An et al. (2013)		
		Phenyltins: 49.04–78.02 ng g <sup>-1</sup> dw				
Babylon shell, <i>Babylonia areolata</i>	Hong Kong	Butyltins: 21.6 µg kg <sup>-1</sup> dw	2012	Ho and Leung (2014a)		
		Phenyltins: 1729.7 µg kg <sup>-1</sup> dw				
<i>Neptunea cumingi</i>	Lianyungang	Butyltins: 37.3 ng Sn g <sup>-1</sup> ww	NA	Zhou et al. (2001)		
		Butyltins: 45.04 ng Sn g <sup>-1</sup> ww	NA			
Various snails	Yantai	Butyltins: 20.2–63.4 ng Sn g <sup>-1</sup> ww	NA	Zhou et al. (2001)		
		Butyltins: 19.8–29.4 ng Sn g <sup>-1</sup> ww	NA			
Various snails	Qingdao	Butyltins: 19.8–29.4 ng Sn g <sup>-1</sup> ww	NA	Zhou et al. (2001)		
		Butyltins: 27.1–32.0 ng Sn g <sup>-1</sup> ww	NA			
Various snails	Tianjin	Butyltins: 27.1–32.0 ng Sn g <sup>-1</sup> ww	NA	Zhou et al. (2001)		





Table 6.3 (continued)

Taxa	Species	Location	Concentration	Year of study	Reference
Crustacean	Unidentified shrimp sample	China (Pearl River)	Butyltins: 16.4 ng g <sup>-1</sup> ww	1997–1999	Zhang et al. (2003)
	<i>Hemigrapsus penicillatus</i>	Tianjin	Butyltins: below detection level	NA	Zhou et al. (2001)
Fish	<i>Orientomyxis koreana</i>	Tianjin	Butyltins: 31.4 ng Sn g <sup>-1</sup> ww	NA	Zhou et al. (2001)
	Various fish species	Thailand	Butyltins: 2.9–16 ng g <sup>-1</sup> ww	1990–1992	Kannan et al. (1995)
	Various fish species	Vietnam	Butyltins: ND–1.7 ng g <sup>-1</sup> ww	1990–1992	Kannan et al. (1995)
	Various fish species	Indonesia	Butyltins: 0.41–19 ng g <sup>-1</sup> ww	1990–1992	Kannan et al. (1995)
	Various fish species	Taiwan	Butyltins: 0.49–18 ng g <sup>-1</sup> ww	1990–1992	Kannan et al. (1995)
	Asian seabass, <i>Lates calcarifer</i>	Bangkok, Thailand	Butyltins: 18.4 ng g <sup>-1</sup> ww	1994	Kannan et al. (1995)
	Red seabream, <i>Pagrus major</i>	Taiwan	Butyltins: 18.5 ng g <sup>-1</sup> dw	1997	Hung et al. (1998)
	Peacock grouper, <i>Cephalopholis argus</i>	Taiwan	Phenyltins: not detected	1997	Hung et al. (1998)
	Asian seabass, <i>Lates calcarifer</i>	Taiwan	Phenyltins: not detected	1997	Hung et al. (1998)
	Black parrotfish, <i>Chlorurus</i> (= <i>Scarus</i> ) <i>cedema</i>	Taiwan	Butyltins: 54.6 ng g <sup>-1</sup> dw	1997	Hung et al. (1998)
	Various fish species	Taiwan	Phenyltins: not detected	1997	Hung et al. (1998)
	Ponyfish, <i>Leiogenathus splendens</i>	Malaysia	Butyltins: 39.8 ng g <sup>-1</sup> dw	1997	Hung et al. (1998)
Lizardfish, <i>Trachinocephalus myops</i>	Taiwan	Phenyltins: not detected	1997–1998	Sudaryanto et al. (2004)	
Unidentified fish sample	Taiwan	Butyltins: 236–2501 ng g <sup>-1</sup> ww (whole body)	1997–1998	Dong et al. (2004)	
Narrow-barred Spanish mackerel, <i>Scomberomorus commerson</i>	China (Pearl River)	Butyltins: 36–159 ng g <sup>-1</sup> ww (muscle)	1997–1998	Dong et al. (2004)	
	Taiwan	3058–11,473 ng g <sup>-1</sup> ww (liver)	1997–1999	Zhang et al. (2003)	
	Taiwan	Butyltins: 11.0–34.0 ng g <sup>-1</sup> ww	2002–2003	Lee et al. (2005)	
		Butyltins: 258.7–1363.9 ng g <sup>-1</sup> dw			

Mammal	Japanese seabream, <i>Pagrus major</i>	Taiwan	Butyltins: 82.1–769.1 ng g <sup>-1</sup> dw	2002–2003	Lee et al. (2005)
	Croceine croaker <i>Larimichthys croceus</i>	Taiwan	Butyltins: 169.1–208.3 ng g <sup>-1</sup> dw	2002–2003	Lee et al. (2005)
	Milkfish, <i>Chanos chanos</i>	Taiwan	Butyltins: 180.2 ng g <sup>-1</sup> dw	2002–2003	Lee et al. (2005)
	Yellow-back seabream, <i>Tauius tumifrons</i>	Taiwan	Butyltins: 310.6–410.0 ng g <sup>-1</sup> dw	2002–2003	Lee et al. (2005)
	Blue-and-gold fusilier, <i>Caesio caeruleaura</i>	Taiwan	Butyltins: 351.1 ng g <sup>-1</sup> dw	2002–2003	Lee et al. (2005)
	Tonguesole, <i>Paraplagusia blochii</i>	Hong Kong	Butyltins: 61.2 µg kg <sup>-1</sup> dw Phenyltins: 2264.6 µg kg <sup>-1</sup> dw	2013	Ho and Leung (2014a)
	<i>Axius tapetinosoma</i>	Beijing	Butyltins: 31.2 ng Sn g <sup>-1</sup> ww	NA	Zhou et al. (2001)
	<i>Trichurus haumela</i>	Beijing	Butyltins: 25.9 ng Sn g <sup>-1</sup> ww	NA	Zhou et al. (2001)
	<i>Engraulis japonicus</i>	Qingdao	Butyltins: 22.3 ng Sn g <sup>-1</sup> ww	NA	Zhou et al. (2001)
	<i>Engraulis japonicus</i>	Yantai	Butyltins: 42.6 ng Sn g <sup>-1</sup> ww	NA	Zhou et al. (2001)
	<i>Muraenesox cinereus</i>	Yantai	Butyltins: 29.6 ng Sn g <sup>-1</sup> ww	NA	Zhou et al. (2001)
	Bryde's whale, <i>Balaenoptera edeni</i>	Thailand	Butyltins: 0.058–0.213 mg kg <sup>-1</sup> ww Phenyltins: 0.028–0.872 mg kg <sup>-1</sup> ww	1997–2002	Harino et al. (2007a)
	False killer whale, <i>Pseudorca crassidens</i>	Thailand	Butyltins: 0.071–4.86 mg kg <sup>-1</sup> ww Phenyltins: 0.022–1.14 mg kg <sup>-1</sup> ww	1997–2002	Harino et al. (2007a)
	Pygmy killer whale, <i>Kogia breviceps</i>	Thailand	Butyltins: 0.141–0.647 mg kg <sup>-1</sup> ww Phenyltins: 0.136–0.413 mg kg <sup>-1</sup> ww	1997–2002	Harino et al. (2007a)
	Short-finned pilot whale, <i>Globicephala macrorhynchus</i>	Thailand	Butyltins: 0.191–0.546 mg kg <sup>-1</sup> ww Phenyltins: 0.058–0.067 mg kg <sup>-1</sup> ww	1997–2002	Harino et al. (2007a)
Sperm whale, <i>Physeter macrocephalus</i>	Thailand	Butyltins: 0.094–0.285 mg kg <sup>-1</sup> ww Phenyltins: 0.106–0.413 mg kg <sup>-1</sup> ww	1997–2002	Harino et al. (2007a)	
Dugongs, <i>Dugong dugon</i>	Thailand	Butyltins: 14–14,468 µg kg <sup>-1</sup> ww Phenyltins: <1–30 µg kg <sup>-1</sup> ww	1997–2002	Harino et al. (2007b)	

NA data not available

of TBT were found in sites near Jurong Island where there are intensive shipping activities (Basheer et al. 2002).

### 6.2.2 Sediment

Concentrations of OTs measured in sediment in Southeast Asia and China during the past 27 years are presented in Table 6.2. Taking Hong Kong as an example, TBT concentrations in sediment fluctuated over time but generally showed a decreasing temporal trend. The TBT concentration in sediment was detected up to 1690 ng g<sup>-1</sup> dry weight (dw) in the early 1990s (Lau 1991). Later, the concentration of TBT in a sediment sample reported by Ko et al. (1995) was detected up to 53,000 ng Sn g<sup>-1</sup> dw (=129,486 ng TBT g<sup>-1</sup> dw). Cheung et al. (2003) detected TBT concentrations up to 560 µg kg<sup>-1</sup> in sediments from Yam O. However, in recent years, only a trace amount of TBT was detected in sediment by the Environmental Protection Department of the Hong Kong SAR Government (EPD 2008).

In China, data on OT contamination in sediment are relatively limited, especially in the northern part of China. Zhang et al. (2003) measured TBT concentrations in the sediment collected from Pearl River Delta and found concentrations ranging from 1.7 to 379.7 ng g<sup>-1</sup> dw, which were lower than in other parts of the world. The highest concentrations of TBT were found in locations close to shipyard and harbours. There was a downward-sloping gradient of TBT concentrations from Pearl River to the estuary, suggesting that the source of TBT was from the intensive riverine shipping activities and sewage discharges, and there was a dilution effect of seawater to the contaminants in the estuary. Huang et al. (2005) investigated the concentrations of OTs in the sediment collected from three harbours along the coast of Southeastern China and found TBT levels between 0.3 and 174.7 ng g<sup>-1</sup> dw. Xiamen Harbour was the most contaminated site among the three harbours, probably because of the discharge of contaminated freshwater and suspended solids from Jiulong River, and such turbid water in Xiamen Harbour resulted in a slower photodegradation rate of TBT therein.

In Taiwan, both butyltin and phenyltin compounds were detected in marine sediment (Meng et al. 2009). Butyltins, in particular TBT, were dominant over phenyltins. TBT was detected as high as 25,300 ng g<sup>-1</sup> wet weight (ww) in a sample collected from the harbour area of Kaohsiung (Jang 2004), whereas in the estuary area of Love River, Kaohsiung, the concentration of TBT could also reach 20,200 ng g<sup>-1</sup> ww. The concentration of TPT was as much as 1811 ng g<sup>-1</sup> dw in the same estuary location (Chi 2004). Tang and Wang (2009) found that a fishing port was a hotspot of TBT contamination in both seawater and sediment compartments, whereas TPT was merely detected in sediment but not in seawater.

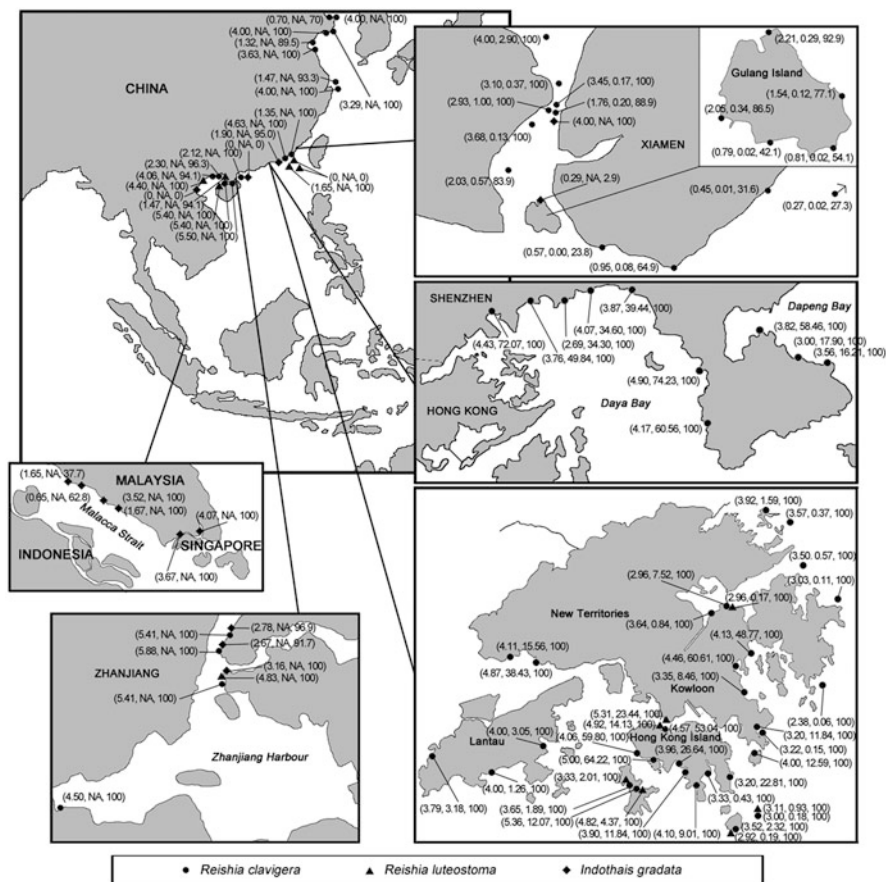
Among several Southeast Asian countries, TBT has been consistently detected in sediment samples collected from Malaysian waters for more than a decade (Tong et al. 1996; Sudaryanto et al. 2004). Harino et al. (2009) reported that TBT concentrations in sediment were higher in Johor Strait among the coastal waters

of Peninsula Malaysia, where there is a major shipping channel. Harino et al. (2012) reviewed the contamination of OTs in Southeast Asia and concluded that higher concentrations of TBT were persistent in sediments found in Malaysia and Thailand, particularly in the coastal areas. Specifically, the highest concentration of TBT at  $1246 \mu\text{g kg}^{-1} \text{ dw}$  was detected in sediment in an industrial area proximal to shipyards in Thailand. This finding echoed the previous study conducted by Kan-Atireklap et al. (1997) in Thailand in which high TBT concentrations, up to  $4500 \text{ ng g}^{-1} \text{ dw}$ , were found in the marine sediment. Phenyltin compounds were also detected at concentrations up to  $35 \mu\text{g kg}^{-1} \text{ dw}$  in sediment in Thailand (Harino et al. 2012). In Vietnam, TBT remained the dominant OT compounds at concentrations ranging from 0.8 to  $28 \mu\text{g kg}^{-1} \text{ dw}$ , and hotspots of TBT contamination include trading ports and coastal sites near industrial areas (Midorikawa et al. 2004).

### 6.2.3 Biota

A wide range of marine organisms, from microalgae to fish, were found to accumulate OTs in their body tissues (Bao et al. 2011; Yi et al. 2012, 2014a, b). Bioconcentration, the direct uptake of OTs from water or sediment, has been clearly shown in marine algae (Yi et al. 2012). OTs can be bioaccumulated along a food chain through diet (Stäb et al. 1996; Yi et al. 2012). TBT has shown to be biomagnified as various studies documented higher TBT concentrations in animals occupying higher trophic levels, such as in marine mammals, fish-eating birds, and humans (Iwata et al. 1997; Kannan and Falandysz 1997). Concentrations of TPT were generally the lowest in seawater, followed by those detected in marine sediment, whereas its concentrations in marine invertebrates and fishes were often higher than those in the two aforementioned environmental compartments (Yi et al. 2012). Previous studies showed that TPT can be biomagnified across lower trophic levels of food chains, and the biomagnification factor of TPT ranged from 2.2 to 5.4 across trophic levels from plankton to fish (Hu et al. 2006; Murai et al. 2008; Fortibuoni et al. 2013). As there were only limited data for TPT concentrations at higher trophic level organisms, the biomagnification of this compound in top predators such as sharks, marine mammals, and seabirds is still unclear at present.

Although OTs were introduced as an antifouling agent to kill the target fouling organisms on ship hulls and submerged mariculture facilities, OTs have been found to induce toxic effects to non-target marine species since the 1970s (Antizar-Ladislao 2008). Being one of the most documented endocrine-disrupting effects of OTs, imposex (i.e., the superimposition of male sexual characteristics—penis and vas deferens—on female gastropods) has been widely observed in females of many gastropod species worldwide. The whelk *Reishia clavigera* (formerly known as *Thais clavigera*; see Claremont et al. 2013) is one of the most extensively used biomonitors of OT contamination in the Asia-Pacific region (Horiguchi et al. 1994; Shim et al. 2000; Leung et al. 2006; Qiu et al. 2011; Ho and Leung 2014b; Ho et al.



**Fig. 6.1** Maps of Southeast Asia showing various studies of imposex status of three whelk species [*Reishia clavigera* (circles), *R. luteostoma* (triangles), and *Indothis gradata* (diamonds)] between 2000 and 2014. Data were extracted from Shi et al. (2005), Leung et al. (2006), Tang et al. (2009), Mohamat-Yusuff et al. (2010), Qiu et al. (2011) and Ho and Leung (2014b). Values inside brackets indicate vas deferens sequence index, relative penis size index, and proportion of imposex-affected females, respectively. NA data not available

2016) because of its high sensitivity towards OTs and its wide distribution across the region. This species could develop imposex at TBT concentrations as low as  $1 \text{ ng l}^{-1}$ , and TPT would have similar effect on this species as did TBT (Horiguchi et al. 1995). This species inhabits intertidal rocky shores from the southeast coast of China to Hokkaido, Japan (Tong 1986). Therefore, such a wide geographic distribution enables field monitoring of this species across the region. Across the Southeast Asian region, Hong Kong (Leung et al. 2006; Qiu et al. 2011), Taiwan (Liu et al. 1997; Hung et al. 2001), and Mainland China (Tang et al. 2009; Xie et al. 2010; Ho and Leung 2014b) have adopted the use of *R. clavigera* as a biomonitor species for monitoring the contamination of OTs in coastal marine environments (Fig. 6.1).

Apart from *R. clavigera*, three other whelk species that originally were placed in the genus *Thais* were also used as biomonitoring species for OT contamination in Southeast Asia. These species appeared to be sensitive towards OTs because the females were observed to be affected by imposex at locations with even slight contamination by OTs (Fig. 6.1): *Reishia luteostoma* (= *Thais luteostoma*) (Ellis and Pattisina 1990; Proud and Richardson 1997; Shi et al. 2005; Leung et al. 2006), *Indothais gradata* (= *Thais gradata*) (Swennen et al. 1997; Shi et al. 2005; Mohamat-Yusuff et al. 2010), and *Menathais tuberosa* (= *Thais tuberosa*) (Mohamat-Yusuff et al. 2011). Several other gastropod species, which belong to families Muricidae and Nassariidae, were also used as biomonitor species for OT contamination: *Nassarius siquijorensis* (Proud and Richardson 1997; Li 2000; Shi et al. 2005), *N. livescens* (Swennen et al. 1997), *N. festivus* (Chan and Morton 2003), and *Ergalatax contracta* (= *Ergalatax contractus*) (Li 2000; Shi et al. 2005). However, they inhabit narrower habitat ranges and are less sensitive towards OTs compared to *R. clavigera*, and hence they have been only used occasionally in regional monitoring studies in the past.

To assess the severity of imposex development on gastropods, two major indices are commonly used. The vas deferens sequence index (VDSI), first developed to quantify the severity of imposex development in *Nucella lapillus* (Bryan et al. 1986; Gibbs et al. 1987), is now widely applied for the assessment of imposex in other gastropod species. A generalized scheme describing the general imposex development in prosobranch gastropods was proposed by Stroben et al. (1995), and this scheme was further developed by Shi et al. (2005) to help assess imposex development in gastropods species other than *N. lapillus*. Cheung et al. (2010) summarised the classification of imposex stages of *R. clavigera* and gave pictorial descriptions on each imposex stage, and Horiguchi et al. (2012) further consolidated the classification of imposex stages of *R. clavigera* by using anatomical and histological observations. The same index has also been used for assessment in *R. luteostoma* (Shi et al. 2005; Leung et al. 2006), *Indothais gradata* (Shi et al. 2005), and *Menathais tuberosa* (Mohamat-Yusuff et al. 2011).

Relative penis size index (RPSI; sometimes relative penis length index is used instead, e.g., in Horiguchi et al. 1994) is another imposex index that is thought to be more objective in terms of measuring the relative sizes of penis between females and males. However, it was criticised that RPSI could vary seasonally (Chan et al. 2008; Tang et al. 2009). RPSI, together with the incidence of imposex (i.e., the proportion of imposex-affected female gastropods), were commonly used to assess the status of imposex in the aforementioned species (Ellis and Pattisina 1990; Leung et al. 2006).

Across the Southeast Asian region, certain large-scale biomonitoring studies have been conducted using *R. clavigera* as the biomonitor for the assessment of OT contamination (Fig. 6.1). A study conducted on the east coast of China, from Dalian to Hainan, showed that OT contamination was still prevalent across the region, especially in areas with intense marine traffic. In a study conducted by Shi et al. (2005) along the east coast of China, the imposex incidence in *R. clavigera* was more than 90% in most of the survey locations. During 2004–2013, all sites in

Hong Kong and Shenzhen recorded 100 % imposex incidence in *R. clavigera* (Leung et al. 2006; Chan et al. 2008; Qiu et al. 2011; Ho and Leung 2014b; Ho et al. 2016), and 5 of 16 sites in Xiamen also showed 100 % imposex incidence (Tang et al. 2008, 2009).

Apart from *R. clavigera*, other gastropods (such as *R. luteostoma* and *I. gradata*) were also used as biomonitors of OT contamination as they exhibit imposex upon exposure to OTs (Fig. 6.1). The disruption of the reproductive system by exposure to OTs has been observed in other gastropods. For instance, the dysfunction of ovaries caused by ovarian dysmaturity and spermatogenesis were observed in the ivory shell *Babylonia japonica* (Horiguchi et al. 2006) and in the abalone *Haliotis gigantea* (Horiguchi et al. 2002).

A growing number of studies started incorporating measurement of tissue concentrations or tissue burdens of OTs as a direct way to quantify the effect of OT contamination in an organism (Table 6.3). However, studies measuring tissue concentrations of TPT and phenyltin compounds were far fewer than those measuring TBT and butyltin compounds. In gastropods, numerous studies have shown that tissue concentrations of OTs increased with decreasing distance to potential sources of OTs, such as piers, shipyards, and fish villages. This relationship has been documented worldwide, not only in Southeast Asia (Leung et al. 2006; Wang et al. 2008; Choi et al. 2009; Guðmundsdóttir et al. 2011).

Tissue concentrations of OTs in gastropods could vary seasonally. Hung et al. (2001) demonstrated that in *R. clavigera* the tissue concentration of butyltins was higher than that of phenyltins in winter, but phenyltin concentration was higher in summer. Qiu et al. (2011) found that the tissue butyltin concentrations were higher in winter than those in summer. Therefore, to understand the temporal dynamics of accumulation and depuration of OTs in gastropods, long-term monthly- or bimonthly-based monitoring surveys are recommended.

Apart from using gastropods as biomonitors, other species from different trophic levels have been quantified for their tissue concentrations of OTs because these compounds can be bioaccumulated along the marine food chain and may affect humans if they consume contaminated seafood. For instance, mussels were often used to quantify the degree of chemical contamination (e.g., metals and synthetic organic chemicals; Leung et al. 2011, 2014a, b) in marine environments. The green-lipped mussel *Perna viridis* had been used to assess the OT contamination in the Strait of Malacca, Malaysia, and it was found that TBT was the predominant compound whereas TPT was not found in the mussel samples (Harino et al. 2009).

Fishes are also often used to assess the OT contamination in the marine environment because of their wide distributions and linkage with human consumption as seafood. In Taiwan, several studies have assessed the OT contamination in various fish species. Dong et al. (2004) investigated the tissue-specific butyltin concentrations in the benthic ponyfish *Leiogenathus splendens* and the lizardfish *Trachinocephalus myops* and found that the concentrations varied seasonally. Lee et al. (2005) showed that certain demersal fishes exhibited high concentrations of TPT in their tissues, including the Japanese seabream *Pagrus major* and the narrow-barred Spanish mackerel *Scomberomorus commerson*. Their team also



demonstrated that the tissue concentrations of OTs in fishes showed seasonal patterns, in which butyltins were dominant in winter and phenyltins dominated in summer. Recently, Ho and Leung (2014a) quantified the amounts of OTs in 11 species of seafood in Hong Kong and found that certain benthic fish species, such as the tonguesole *Paraplagusia blochii*, could have higher tissue concentrations of OTs than other species, posing a higher health risk to humans at an average level of fish consumption.

OTs, in particular TPT, could induce immunotoxic effects in marine mammals and humans (Nakayama et al. 2009; Yi et al. 2012). With a large log  $K_{ow}$  value, ranging from 3.0 to 5.0 (Yi et al. 2012), it has been demonstrated that both TBT and TPT can be biomagnified through the food chain in coastal ecosystems to a certain extent (Strand and Jacobsen 2005; Hu et al. 2006), although the biomagnification of TPT through the food chain at higher trophic levels is still not clearly demonstrated.

For measuring OTs in marine mammals, the liver is the most commonly used target tissue type because concentrations of total butyltins in the liver were demonstrated to be much higher than those in other tissues such as muscle and blubber (Iwata et al. 1997). Nakayama et al. (2009) quantified the concentrations of various OTs in the liver of the finless porpoise *Neophocaena phocaenoides* collected in Hong Kong waters. The results showed that both butyltins and phenyltins concentrations were 10- to 20-fold higher than those measured in Japan, indicating that Hong Kong has been heavily contaminated with these OTs. It had been showed that OTs could increase the susceptibility of parasitic infection in the porpoises (Nakayama et al. 2009). Tanabe et al. (1998) quantified the concentrations of OTs in the livers of the finless porpoise from China, and the long-snouted spinner dolphin (*Stenella longirostris*) and Fraser's dolphin (*Lagenodelphis hosei*) from the Philippines, and reported that the highest tissue concentration was 890 ng g<sup>-1</sup> ww. Choi et al. (2013) investigated the temporal variation (2003–2010) of TBT concentrations in the liver of the finless porpoise *Neophocaena asiaeorientalis* from the Yellow Sea near Qingdao, and found that the highest total butyltin concentration was 1432 ng g<sup>-1</sup> ww, but that the concentrations were declining over the years.

Harino et al. (2007a, b) measured the concentrations of OTs in marine mammals, including whales and dugongs from Thailand, and found that tissue-specific butyltin concentrations were generally higher than those of phenyltins. Most OTs accumulated in the liver rather than other parts of the body such as blubber, lung, kidney, and muscle. In particular, the false killer whale from Phuket had the highest concentrations of butyltin compounds, up to 5 mg kg<sup>-1</sup> ww.

### 6.3 Conclusions and Perspectives

The published data clearly showed that contamination of OTs in the coastal marine environments of Southeast Asia was undoubtedly severe. However, the current legislations in this region were far from adequately protective of the marine ecosystem. For those countries or areas with implementation of partial restrictions

on the use of OT-based antifouling paints (such as Hong Kong and Taiwan), more stringent regulations should be enacted locally or nationally to strengthen controls on the use and release of these chemical contaminants into the marine environment. For those countries or areas currently without any such restrictions, a comprehensive baseline survey on the contamination status of OTs should be conducted in different compartments of the marine environment, including seawater, sediment, and biota, with a view to fully determining the severity of OTs contamination in that region, evaluating the ecological risks associated with OTs, and making appropriate management strategies to better protect the ecosystem. The countries or areas in this region, especially those that already have had reasonable amounts of study on OTs, should be an integral part of information exchange, knowledge transfer, and research collaboration to jointly tackle such a pressing environmental issue in the region.

The industry is now phasing out the use of OT-based antifouling paints and searching for promising alternatives of booster biocides such as Irgarol 1051, copper pyrithione, zinc pyrithione, and triphenylborane. However, these synthetic booster biocides have been accused of being toxic to a variety of non-target marine organisms (Yebra et al. 2004; Zhang et al. 2008; Okamura et al. 2009; Bao et al. 2012). There are also uncertainties regarding their environmental impacts, including their bioaccumulation potential and synergistic interactions with other chemical pollutants (Yebra et al. 2004; Bao et al. 2013, 2014). Apart from comprehensively studying their environmental behaviours, the development of naturally biodegradable biocides and self-cleaning surfaces (e.g., trypsin-based coatings; Shi et al. 2011) would also help strike a balance between environmental protection and antifouling performance.

With the advances in technology, several molecular methods have been developed to help assess the severity of effects of OTs on biota, particularly on imposex development on marine gastropods, including gene and protein expression assays (Horiguchi et al. 2007). Recently, the use of the transcriptomic approach to reveal the toxic mechanisms of chemicals towards marine organisms has become popular and economical. For example, two transcriptomic libraries of *Reishia clavigera* (Ho et al. 2014; Ip et al. 2016) and *Perna viridis* (Leung et al. 2014a), which are common biomonitors in Asia, have been developed and made available in the GenBank for reference. These genomic resources could help environmental researchers to better understand the underlying molecular toxic mechanisms of OTs on marine organisms.

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