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Organotin levels in bivalves in Southeast Asia

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Abstract—The distribution of organotin compounds in bivalves collected from Vietnam, Thailand and Indonesia was studied in order to examine the present status of contamination on marine environment. The bivalves such as clams *Meretrix* spp., green mussels *Perna viridis*, and arkshells *Anadara granosa* and *A. antiquata* were purchased at each local market or collected from each coast. Six organotin compounds, monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), monophenyltin (MPT), diphenyltin (DPT) and triphenyltin (TPT) were analyzed in these samples using GC-MS. Concentrations of TBT in bivalves specimens collected from Vietnam, Thailand and Indonesia were in the range of 0.37–56 ng g^{-1} wet wt., 0.83–16 ng g^{-1} wet wt. and 9.7–37 ng g^{-1} wet wt., respectively. Among 12 Vietnamese sites, average concentration of TBT (47 ng g^{-1} wet wt.) in clams from Cua Luc was the highest level, and the ratio of TBT to ΣBTs in this site was 86%. As Cua Luc is an industrial town having large international trading port, continuous input of TBT from vessels might be indicated. Concentrations of MPT in clams from Vietnam ranged from 0.16 to 11 ng g^{-1} wet wt., and those of DPT and TPT in clams from Vietnam and MPT, DPT and TPT in bivalves from Thailand and Indonesia were close to the detection limit. The present study indicated that organotin compounds in bivalves from Vietnam, Thailand and Indonesia were fallen in the range of levels reported throughout the world.

Key words: organotin compounds, TBT, TPT, Southeast Asia

Introduction

Organotin compounds are regarded as disruptive contaminants in this age. These compounds have been used as polyvinyl chloride stabilizers, industrial catalysts, wood preservatives and various biocides since 1960s. Particularly in marine environment, organotin compounds such as TBT and TPT have been used as antifouling agents in paints applied for ship bottom and aquaculture net, resulted in serious marine pollution problems that have deleterious effect to non-target marine organisms such as shell deformation for oysters (Alzieu et al. 1989) and imposex for gastropods (Bryan and Gibbs 1991).

Because of high toxicity of organotin compounds, the application of TBT-based antifouling paints was banned in the late 1980s and early 1990s on ships less than 25 m in total length in Europe, USA, Canada, Australia, New Zealand, Japan and Hong Kong (Stewart 1996, Evans et al. 1996, Horiguchi et al. 1994, Ko et al. 1995). Furthermore, global prohibition on the application of organotin compounds as biocides in antifouling systems on ships by January 1, 2003, and a complete prohibition of the presence of organotin compounds by January 1, 2008, were agreed by the International Maritime Organization (IMO) (Champ 2000). These regulations are the first step for prohibiting the use of TBT-based antifouling paints for Southeast Asian countries joining IMO. On the other hand, marine pollution caused by organotin compounds in Southeast Asia has been already reported until now, although it is limited. Most of Southeast Asian countries have achieved rapid economic development, and developed a large-scale trade, anticipating marine pollution through busy ship navigations. More fundamental data on organotin levels in Southeast Asia where had experienced decades of unrestricted use of TBT are required to understand the transition of organotins pollution before and after the regulation, and to assess

the regulation accurately. In this study, organotin levels in bivalves collected from Vietnam, Thailand and Indonesia during 2002–2003 were measured in order to examine the distribution of these compounds in Southeast Asia, particularly examined in Vietnam in detail.

Materials and Methods

Sample collection

The clam *Meretrix* spp. samples were purchased at each local market in northern Vietnam (Tra Co, Cua Luc, Do Son, Ba Lat, Sam Son, and Cua Lo) and central Vietnam (Dong Hoi and Da Nang), and collected from coastal area of southern Vietnam (Ho Chi Minh, Tien Giang, Ben Tre and Bac Lieu). (Fig. 1), and also purchased at local market in Bangkok, Thailand. The green mussels *Perna viridis* were collected from the coastal area of Bangkok in Thailand and Jakarta in Indonesia. The arkshells *Anadara granosa* were purchased at local market in Bangkok, Thailand, arkshells *A. antiquata* were collected from coastal area in Jakarta, Indonesia. Shell lengths of clams, green mussels and arkshells were in the range of 41.3–86.3 mm, 48.4–79.8 mm and 24.8–37.5 mm, respectively. The sampling was conducted in northern Vietnam in March 2002, in central Vietnam in August 2002, in southern Vietnam in November 2002 and in Thailand and Indonesia in March 2003. All samples were brought back to Japan in a cold box and frozen in −20°C until chemical analysis.

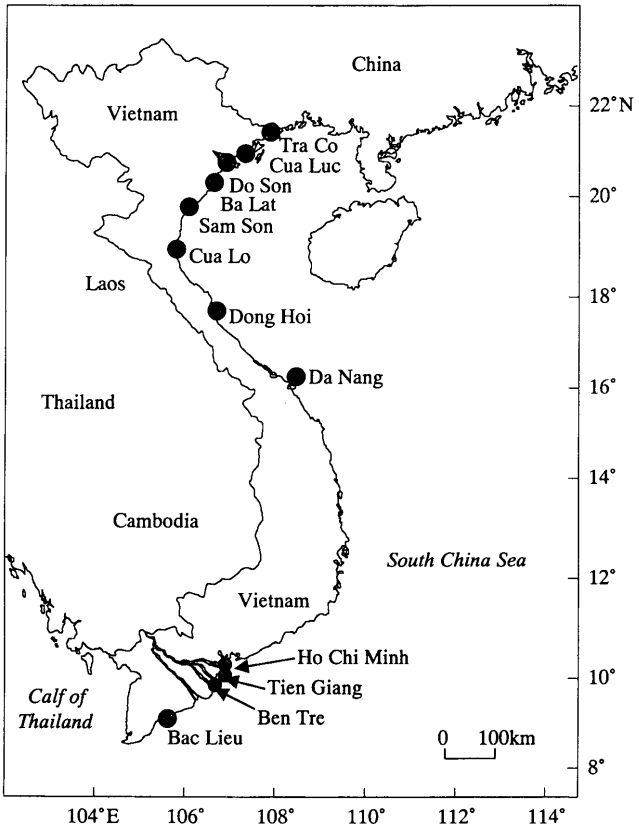

Fig. 1. Sampling sites in Vietnam.

Analytical procedure

Butyltin (BT) and phenyltin (PT) compounds in bivalves samples was analyzed following by Iwamura et al. (2000) with some modification.

About 2–5 g of the homogenate soft tissues of bivalves samples were taken in a centrifuge tube and 100 μl of mixed acetone solution including 1 μ/ml of each monobutyltin trichloride (MBTCl)-d_9, dibutyltin dichloride (DBTCl)-d_{18}, tributyltin monochloride (TBTCl)-d_{27}, monophenyltin trichloride (MPTCl)-d_5, diphenyltin dichloride (DPTCl)-d_{10}, and triphenyltin monochloride (TPTCl)-d_{15} was added to the centrifuge tube as a surrogate standard. The mixture was extracted with 25 ml of 1 M HCl–methanol/ethyl acetate (1/1) by shaking for 10 min. After centrifugation for 10 min, the residue was extracted and centrifuged again. The combined supernatants and 100 ml of saturated NaCl solution were transferred to a separatory funnel. The analytes were extracted twice using 30 ml of ethyl acetate/hexane (3/2) solution. A hundred milliliters of hexane was mixed with the combined organic layers and kept standing for 20 min. After removal of the aqueous layer, the organic layer was dried with anhydrous Na_2SO_4 and was concentrated up to trace level. The analytes were diluted with 5 ml of acetic acid-sodium acetate buffer (pH 5.0) and ethylated using 1 ml of 10% NaBEt4. The lipids were saponificated with 40 ml of 1 m KOH–ethanol solution by shaking for 1 h. Twenty-five milliliters of distilled water and 40 ml of hexane were added to the solution and the mixture was shaken for 10 min. After removal of the aqueous layer, the analytes were again extracted using 40 ml of hexane. The combined organic layers were dried with anhydrous Na_2SO_4. After being concentrated up to 1 ml, the solution was cleaned by a florisil Sep-Pak column (Waters Association Co. Ltd.) The analytes were eluted with 5% diethyl ether/hexane, and TeBT-d_{36} and TePT-d_{20} were then added as an internal standard. The final solution was concentrated up to 0.2 ml.

A Hewlett-Packard 6890 series gas chromatography equipped with a mass spectrometry (5973 N) was used for analysis of OTs with selected ion monitoring. The separation was carried out in a capillary column coated with 5% phenyl methyl silicone (J&W Scientific Co., 30 m length×0.25 mm i.d., 0.25 μm film thickness). The column temperature was held at 60°C for the first 2 min, then increased to 130°C at 20°C/min, to 210°C at 10°C/min, to 260°C at 5°C/min, and to

300°C at 10°C/min. Finally, column temperature was held 300°C for 2 min. Interface temperature, ion source temperature, and ion energy were 280°C, 230°C, and 70 eV, respectively. Selected ion monitoring was operated under this program. Splitless injection (1 μl) of the sample was employed. The concentrations of OTs in this study are expressed as Sn^{4+} on a wet weight basis.

In order to examine the quality of data obtained by above analytical procedure, recovery test was carried out. Recoveries of MBT, DBT, TBT, MPT, DPT and TPT were 108±11, 96±10, 92±9, 119±8, 94±10 and 86±20%, respectively. The detection limits of each organotin compounds for a signal-to-noise ratio of 3 were in the range of 0.04–0.1 ng g^{-1} wet wt.

Results and Discussion

Distribution of OTs in clams from Vietnam

Butyltin compounds were widely distributed along the Vietnamese coast (Fig. 2). Concentrations of MBT, DBT, and TBT in clam *Meretrix* spp. from all sites of Vietnam were in the range of 0.1 to 44, 0.3 to 10, and 0.4 to 56 ng g^{-1} wet wt., respectively. Total butyltin (ΣBts) concentrations were in the range of 2.1–63 ng g^{-1} wet wt., these were much lower in clams from southern Vietnam at 2.9–17 ng g^{-1} wet wt. Sampling points in all 4 sites in southern Vietnam are around coastal aquaculture areas. No or slight input of TBT from aquaculture net might be supposed. The highest concentration of TBT was found in Cua Luc and the value was 47 ng g^{-1} wet wt. In all the other sites of Vietnam, concentrations of TBT were in the range of 0.41–6.8 ng g^{-1} wet wt. As to composition of butytin compounds, the highest ratio of MBT among butyltin derivatives was found in clams from Som Son, Dong Hoi, Da Nang, Tien Giang and Bac Lieu, while TBT was the highest ratio in the other 7 sites. The ratio of TBT in clams from Cua Luc where the highest concentration was observed was 86%. Cua Luc is an industrial area with a big international trading port. Many vessels including large-hulled vessels from various countries therefore moor there. These findings suggest that a major source of TBT in Cua Luc was due to the antifouling paint leaching from these vessels. Concentration of MBT were higher in Dong Hoi (29 ng g^{-1} wet wt.) and Da Nang (28 ng g^{-1} wet wt.), which is fishing port area and international trading port area, respectively. In both Dong Hoi and Da Nang, the ratio of MBT was predominant among butyltin compounds.

Phenyltin compounds were also widely distributed in Vietnamese coast. Concentrations of MPT, DPT, and TPT in clams from all sampling sites were in the range of 0.2 to 11, <0.1 to 0.7, and <0.1 to 3.1 ng g^{-1} wet wt., respectively. The trend of distribution of phenyltin compounds was similar to those of butyltin compounds. TPT level was the highest in Cua Luc. Higher level and higher ratio of MPT were found in Dong Hoi and Da Nang. This might suggest that the coastal marine environment in Dong Hoi and Da Nang had been contaminated by much TBT in the past. Phenyltin concentrations in most of the sampling sites were close to the detection limit.

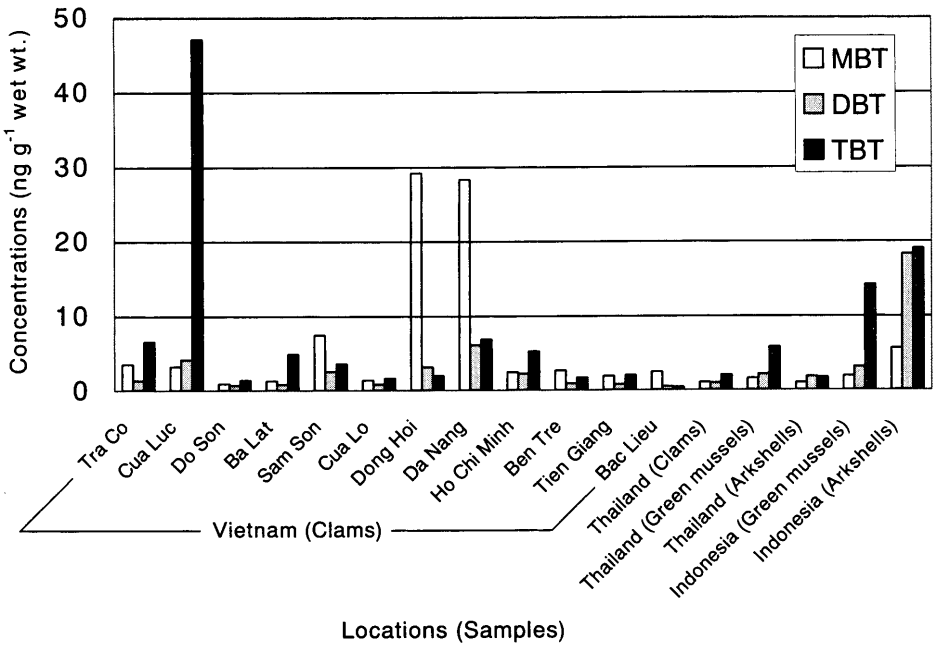

Fig. 2. Concentrations of butyltin compounds in bivalves samples from Vietnam, Thailand and Indonesia.

Table 1. Organotins levels in bivalves from Southeast Asian countries.

Location	sampling year	samples	Concentrations (ng g^{-1} wet wt.)						References
			MBT	DBT	TBT	MPT	DPT	TPT	
Vietnam	1998	green mussels (*Perna viridis*)	<2.1–3.3	<0.86–19	2.1–64	—	—	—	Sudaryanto et al. 2000
	2002	clams (*Meretrix* spp.)	0.08–44	0.34–10	0.37–56	0.16–11	<0.1–0.69	<0.1–3.1	This study
Cambodia	1998	green mussels (*Perna viridis*)	<2.0–25	<0.98–37	2.4–88	—	—	—	Sudaryanto et al. 2000
Thailand	1994	green mussels (*Perna viridis*)	<3–45	1–66	5–200	—	—	—	Kan-Atireklap et al. 1997
	1995	green mussels (*Perna viridis*)	<3–42	1–80	3–680	—	—	—	Kan-Atireklap et al. 1997
	2003	green mussels (*Perna viridis*)	0.93–2.5	0.57–5.1	0.83–16	0.11–0.25	0.23–0.28	<0.06–0.10	This study
	2003	clams (*Meretrix* spp.)	0.85–1.2	0.63–1.1	0.91–3.2	0.15–0.20	0.19–0.21	<0.04–0.08	This study
	2003	arkshells (*Anadara granosa*)	0.77–1.2	1.3–3.2	1.3–2.1	0.07–0.15	0.21–0.35	<0.04–1.8	This study
Malaysia	1991–1992	cockles (*Anadara granosa*)	—	—	<0.5–5.6	—	—	—	Tong et al. 1996
	1992	green mussels (*Perna viridis*)	—	—	14.2–23.5	—	—	—	Tong et al. 1996
	1992	soft-shelled clams (*Paphia* sp.)	—	—	3.7±2.0	—	—	—	Tong et al. 1996
	1998	green mussels (*Perna viridis*)	<2.6–74	<1.0–160	3.5–730	—	—	—	Sudaryanto et al. 2000
Indoneis	1998	green Mussels (*Perna viridis*)	1.5–13	<0.58–14	2.2–38	—	—	—	Sudaryanto et al. 2000
	2003	green mussels (*Perna viridis*)	1.6–2.1	2.9–3.4	14–14	0.16–0.36	0.33–0.45	0.11–0.14	This study
	2003	arkshells (*Anadara antiquata*)	2.7–11	9.5–35	9.7–37	0.13–0.15	0.18–0.28	0.08–0.13	This study
Phillippines	1994–1997	green mussels (*Perna viridis*)	<3–51	<1–100	<1–640	—	—	—	Prudente et al. 1999
	1997–1998	green mussels (*Perna viridis*)	<2.0–15	<1.3–19	0.8–47	—	—	—	Sudaryanto et al. 2000

It is well known that TPT is more stable than TBT in biological samples (Fent and Hunn, 1991). Nevertheless, the levels of TBT in clams were also higher than TPT levels, suggesting a greater TBT input to the aquatic environment in comparison with TPT.

Concentrations of OTs in bivalves samples from Thailand and Indonesia

Butyltin levels in bivalves samples from Thailand and Indonesia were shown in Fig. 2. Concentrations of MBT, DBT and TBT in clams *Meretrix* spp. from Thailand were in the range of 0.85–1.2, 0.63–1.1 and 0.91–3.2 ng g^{-1} wet wt., respectively. These levels were same trend with those in lower contaminated site surveyed in Vietnam such as Do Son, Cua Lo and Tien Giang. Butyltin levels of arkshells *Anadara granosa* from Thailand were also similar to these levels. In green mussels *Perna viridis* from Thailand, concentration of TBT was slightly higher than these level at average 5.8 ng g^{-1} wet wt. Mean concentrations of TBT in green mussels *Perna viridis* and arkshells *A. antiquata* from Indonesia were 14 and 19 ng g^{-1} wet wt., respectively. These levels of bivalves from Indonesia were higher than those from uncontaminated sites in Vietnam and from Thailand and lower than those from a contaminated site (Cua Luc) in Vietnam. Composition of butyltin compounds in bivalves from Indonesia were higher in the order of TBT>DBT>MBT, suggesting fresh input of TBT to the coastal environment in Indonesia. Concentrations of phenyltin compounds were much lower than butyltin compounds as well as the case of Vietnam.

Status of organotins levels in Southeast Asia

The levels of organotin compounds in bivalves from coastal area of Southeast Asia reported previously were summarized in Table 1. Butyltin compounds were widely distributed in Southeast Asia. On the other hand, phenyltin compounds were distributed slightly compared with butyltin compounds. Concentrations of TPT in the muscle of a few fish species in Southeast Asia were also reported at below the limit of detection of 20 ng g^{-1} wet wt. (Kannan et al. 1995a). In this study, TPT levels in bivalves samples collected from Vietnam, Thailand and Indonesia were in the range of n.d. 23.1 ng g^{-1} wet wt. Organotin pesticide formulations such as 'Brestan' and 'Aquatin' (TPT) are used in coastal aquaculture in Southeast Asia to eradicate snails in fish ponds (Kannan et al. 1995b). Slight detection of phenyltin compounds in Southeast Asia might indicate that there are such slight use of TPT as antifouling paint for domestic and/or international ships in Southeast Asian countries.

Higher concentrations of TBT were reported in green mussels from Thailand in 1995 at 3–680 ng g^{-1} wet wt. (Kan-

Atireklap et al. 1997), Malaysia in 1998 at 3.5–730 ng g^{-1} wet wt. (Sudaryanto et al. 2000) and Philippines in 1994–1997 at <1–640 ng g^{-1} wet wt. (Prudente et al. 1999). Compared with these data, lower levels of TBT were found in Vietnam, Cambodia and Indonesia. Higher level of organotins was locally found in some locations in each country. Most of these locations were active maritime areas such as big harbor, international port and shipyard. Organotin levels were seemed to depend on mainly by distance from potential sources of TBT such as antifouling paint in ship and aquaculture net, and water exchange capacity. And TBT was dominant species in most samples from Southeast Asia. These results indicate that fresh input of TBT from antifouling paint on ship was occurred in the marine environment. To our knowledge, monitoring of organotin compounds in Southeast Asian countries in 2003 is first time since the usage of TBT-based antifouling paint has been restricted globally. Butyltins levels in bivalves samples collected from Vietnam in 2002, and Thailand and Indonesia in 2003, obtained in this study, were at the same level or lower compared with those of previous studies. This indicates that organotin compounds have been still remained in the coastal area of Southeast Asian countries. In future when the use of TBT-based antifouling paint has restricted, it is required to observe the transition of organotins levels in order to assess the regulation accurately.

Comparison of TBT levels with TARL

A tolerable daily intake (TDI) of TBT was derived to be 0.25 μg/kg body weight/day for TBTO by Penninks (1993), this value is generally accepted and referred to the previous studies (Kannan and Falandysz 1997, Robinson et al. 1999, Belfroid et al. 2000). Belfroid et al. (2000) calculated tolerable average residue levels (TARL) from TDI based on the formula as described below.

$$\text{TARL} = (\text{TDI} \times 60\,\text{kg body weight}) / (\text{average daily seafood consumption})$$

Average daily seafood consumptions in Vietnam, Thailand and Indonesia estimated by FAO, 1998 were 34.5, 71.0 and 41.6 g/day, respectively (Belfroid et al. 2000). Based on these data, the TARL for seafood in Vietnam, Thailand and Indonesia were calculated to be 435, 211 and 360 ng g^{-1} wet weight as TBTO, 87, 42 and 72 ng g^{-1} wet weight as Sn for an average person weighing 60 kg. The TBT levels in bivalves samples collected from Vietnam (0.37–56 ng g^{-1} wet wt.), Thailand (0.83–16 ng g^{-1} wet wt.) and Indonesia (9.7–37 ng g^{-1} wet wt.) in this study were lower than the TARL.

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References

Alzieu, C. M., Sanjuan, J., Deltheil, J. P. and Borel, M. 1989. Monitoring and assessment of butyltins in Atlantic coastal waters. Mar. Pollut. Bull. 20: 22–26.

Belfroid, A. C., Purperhart, M. and Ariese, F. 2000. Organotin Levels in Seafood, Mar. Pollut. Bull. 40: 226–232.

Bryanm G. W. and Gibbs, P. E. 1991. Impact of low concentrations of tributyltin (TBT) on marine organisms: a review. In Metal Ecotoxicology Conenpts and Applications, Newman M. C. and McIntosh, A. W. (ed.), pp. 323–361, Lewis Publishers.

Champ, M. A. 2000. A review of organotin regulatory strategies, pending actions, related costs and benefits. Sci. Total. Environ. 258: 21–71.

Evans, S. M., Evans, P. M. and Leksono, T. 1996. Widespread recovery of dogwhelks *Nucella lapillus* (L.) from tributyltin contamination in the North Sea and Clyde Sea. Mar. Pollut. Bull. 32: 263–269.

Fent, K. and Hunn, J. 1991. Phenyltins in water, sediment, and biota of freshwater marinas. Environ. Sci. Technol. 25: 956–963.

Horiguchi, T., Shiraishi, H., Shimizu, M. and Morita, M. 1994. Imposex and organotin compounds in *Thais clavigera* and *T. bronni* in Japan. J. Mar. Biol. Assoc. UK. 74: 651–669.

Iwamura, T., Kadokami, K., Jin-ya, D. and Tanada, K. 2000. Determination of organotin compounds in biological samples using ethyl derivatization and GC/MS. Anal. Sci. 49: 523–528.

Kan-Atireklap, S., Tanabe, S., Sanguansin, J., Tabucanon, M.S. and Hungspreugs, M. 1997. Contamination by butyltin compounds and organochlorine residues in green mussel (*Perna viridis*, L.) from Thailand coastal waters. Environ. Pollut. 97: 79–89.

Kannan, K., Tanabe, S. and Tatsukawa, R. 1995a. Phenyltin residues in horseshoe crabs, *Tachypleus tridentatus* from Japanese coastal waters. Chemosphere. 30, 925–932.

Kannan, K., Tanabe, S., Iwata, H. and Tatsukawa, R. 1995b. Butyltins in muscle and liver of fish collected from certain Asian and Oceanian countries. Eviron. Pollut. 90: 279–290.

Kannan, K. and Falandysz, J. 1997. Butyltin residues in sediment, fish, fish-eating birds, harbor porpoise and human tissues from the Polish coast of the Baltic Sea. Mar. Pollut. Bull. 34: 203–207.

Ko, M. M. C., Bradley, G. C., Neller, A. H. and Broom, M. J. 1995. Tributyltin contamination of marine sediments of Hong Kong. Mar. Pollut. Bull. 31: 249–253.

Prudente, M., Ichihashi, H., Kan-atireklap, S., Watanabe, I. and Tanabe, S. 1999. Butyltins, organochlorines and metal levels in green mussel, *Perna viridis* L. from the coastal waters of the

Philippines. Fish. Sci. 65: 441–447.

Robinson, S., Volosin, J., Keithly, J. and Cardwell, R. 1999. Comment on: Butyltin residues in sediment, fish, fish-eating birds, harbour porpoise and human tissues from the Polish coast of the Baltic Sea (Kannan and Falandysz 1997). Mar. Pollut. Bull. 38: 57–61.

Stewart, C. 1996. The efficacy of legislation in controlling tributyltin in the marine environment. In Tributyltin: case study of an environmental contaminant. de Mora, S. J. (ed.), pp. 264–297, Cambridge University Press, Cambridge.

Sudaryanto, A., Takahashi, S., Tanabe, S., Muchtar, M. and Razak H. 2000. Butyltins residues in green mussels (*Perna viridis*) from Indonesian coastal waters and other Asian countries. Mussel Watch: Marine pollution monitoring in Asian waters, edited by Tanabe, S. 137–144.

Tong, S. L., Pang, F. Y., Phang, S. M. and Lai, H. C. 1996. Tributyltin distribution in the coastal environment of peninsular Malaysia. Environ. Pollut. 91: 209–216.