Butyltin Contamination in Mussels from Vietnam and Other Asian Developing Countries

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Abstract: Butyltin compounds (BTs) including tributyltin (TBT) and its breakdown products and total tin (∑Sn) were determined in green mussels (*Perna viridis*) from Vietnam and other Asian developing countries such as Cambodia, China (Hong Kong and southern China), Malaysia, India, Indonesia, Philippines to elucidate the contamination status, distribution and possible sources and to assess the risks on aquatic organisms and humans. BTs were detected in green mussels collected from all the sampling locations investigated, suggesting widespread contamination of BTs along the coastal waters of Asian developing countries. Among butyltin derivatives, tributyltin (TBT) was the predominant compound, indicating its ongoing usage and recent exposures in Asian coastal waters. Higher concentrations of BTs were found in mussels collected at locations with intensive maritime activities, implying that the usage of TBT as a biocide in antifouling paints was a major source of BTs. In addition, relatively high concentrations of BTs were observed in mussels from aquaculture areas in Hong Kong and Malaysia, as it has been reported in Thailand. With the recent improvement in economic status in Asia, it is probable that there will be an increase in TBT usage in aquaculture. Although contamination levels were generally low in mussel samples from Vietnam and most of the Asian developing countries, some of those from polluted areas in Hong Kong, India, Malaysia, Philippines and Thailand revealed levels comparable to those in developed nations. Furthermore, the concentrations of TBT in some mussels from polluted areas exceeded the threshold for toxic effects on organisms and estimated tolerable average residue levels as seafoods for human consumption. A significant correlation was observed between the concentrations of ∑BTs and ∑Sn in mussels, and ∑BTs were made up mostly 100% of ∑Sn in mussels taken from locations having intensive maritime/human activities. This suggests that anthropogenic BTs is the major source of tin accumulation in mussels.

Introduction

The rapid economical growth in Asian developing countries has resulted in increasing production and usage of toxic chemicals such as heavy metals, pesticides, polychlorinated biphenyls, aromatic hydrocarbons and orgaometallic compounds, which are of great implications on human and ecosystem health [1-7].

Butyltin compounds (BTs), one of the representative organometallic compounds, have been extensively used for industrial and agricultural purposes such as polyvinyl chloride (PVC) stabilizers, industrial catalysts, wood preservatives and various biocides since the 1960s. Particularly, toxic tributyltin (TBT) has been used as an effective antifouling agent in paints applied for pleasure boats, large ships and vessels, harbor structures and on aquaculture nets. Aquatic pollution resulting from the usage of TBT has been of great concern in many counties due to its effects on non-target marine organisms. It has been documented that some mollusks such as oysters [8] and prosobranch gastropods [9-11] are quite susceptible to toxic effect of tributyltin (TBT). Chronic toxic effects of TBT such as anomalies in shell calcification of oysters and 'imposex phenomenon' in gastropods were reported to occur at a few ng TBT/L levels [9, 10 12]. The toxic concentrations of TBT to embryonic and early life stages of aquatic organisms lie in the range of a few μg/L or even lower [9, 12, 13]. It has been reported that TBT can disrupt the endocrine system in some mollusks [14].

Concerns over the ecotoxicological impact of TBT led to the restriction of TBT-based antifouling paints in most developed countries since the 1980s. However, in developing countries in Asia, no restriction has so far been imposed on the usage of TBT. Although large number of studies on butyltin pollution has been conducted in developed nations, information on contamination status in Asian developing countries is still meager. No investigation regarding organotin pollution has yet been conducted in Vietnam. On the other hand, several studies have reported the contamination of BTs in some Asian and Oceanian countries [2, 15-22]. These observations indicate the expansion of butyltin contamination even in Asian developing countries. It should also be noted that an increasing demand for antifouling paints was predicted in the Asia-Pacific region [23]. Considering the above facts, it is necessary to elucidate the present status of butyltin contamination in coastal waters of Asian developing countries including Vietnam and to predict future trend.

Bivalves such as mussels have been used as bioindicators for monitoring trace toxic substances in coastal waters [24]. In the Asia-Pacific region, the green mussel (*Perna viridis*) are widely distributed along the coasts, and is also a commercially valuable seafood in this region [25]. Since 1994, our group has conducted monitoring studies in connection with the Asia-Pacific Mussel Watch Program (APMW), under the International Mussel Watch, which aimed at monitoring the marine pollution in Asian coastal waters using mussels as bioindicators [4, 5, 26]. The APMW was a collaborative project of scientists from various Asian countries such as Vietnam, Cambodia, Hong Kong, India, Indonesia, Korea, Malaysia, Philippines, Thailand and Japan. Our earlier studies were conducted in Thailand, Philippines and India during 1994-1997 [27-29]. These studies detected butyltin residues as well as organochlorine pesticides and polychlorinated biphenyls (PCBs) in mussels from almost all the sampling locations [5].

The present study was conducted likewise as a part of the APMW project to further elucidate the present status of contamination by BTs in coastal waters of remaining Asian developing countries including Vietnam which were not included in earlier reports. In addition, this study was aimed at reviewing distribution of BTs in the Asia-Pacific region. Concentrations of BTs including TBT and its breakdown products, di- (DBT) and monobutyltin (MBT), were determined in the whole soft tissue of green mussels (*Perna viridis*) collected along the coastal waters of Cambodia, China (Hong Kong and southern China), India, Indonesia, Malaysia, Philippines and Vietnam during 1997 to 1999. The results of this study together with the previous ones were compared with those reported in various regions of the world to delineate the magnitude of contamination in Asian developing countries. The data obtained in this project was also evaluated in terms of possible impacts on aquatic organisms and risks to human health. In addition, total tin ($\sum$Sn) concentrations were determined in the mussel samples. Relationship of concentrations between BTs and $\sum$Sn was also examined to verify the anthropogenic input of tin compounds.

Materials and Methods

Samples

Green mussels (*Perna viridis*) were collected from the coastal waters of Vietnam, Cambodia, China (Hong Kong and southern China), India, Indonesia, Malaysia and Philippines during 1997 to 1999 (Table 1 and Fig. 1). The sampling locations included urban population centers, commercial and fishery harbors, marinas, recreational boating and marine aquaculture areas as well as pristine remote locations. The details of the sampling sites are given in Table 1. More than 20 mussels were collected from each sampling location and adhering matrices were removed in the field. Samples were stored in polyethylene bags, kept in a cooler box with ice or dry ice and then immediately kept in a deep freezer. In the laboratory, the frozen green mussel samples were thawed and employed for biometric measurement. After shucking, the whole soft tissues of green mussels were pooled, homogenized, transferred into clean glass bottles and frozen at -20°C until chemical analysis. Biometric data of green mussel samples are shown in Table 1.

Chemical Analysis

BTs were analyzed following the methods described by Kan-atireklap et al. [27]. BTs were detemined and quantified by a gas chromatograph (Hewlett-Packard 5890 Series II) equipped with flame photometric detector (GC-FPD). Fused silica capillary column (30 m length x 0.25 mm i.d., 0.25 μm film thickness) coated with DB-1 (J&W Scientific Co.,

Table 1. Sampling locations, date and biometric data of green mussels (*Perna viridis*) collected from coastal waters of Vietnam, Cambodia, China, India, Indonesia, Malaysia and Philippines.

Location	Site code[a]	Sampling date	*n*	Shell length (mm)	Tissue weight (g wet wt)	Area description
VIETNAM						
Cat ba, Cat Hai Province	VNCHCB-1	09/29/97	38	13 (9-16)	26 (13-53)	floating habitat, urban area
Cat ba, Cat Hai Province	VNCHCB-2	10/27/97	34	9 (5-13)	13 (3-39)	floating habitat, urban area
Cat ba, Cat Hai Province	VNCHCB-3	10/27/97	8	12 (10-13)	24 (15-31)	floating habitat, urban area
Cat ba, Cat Hai Province	VNCHCB-4	10/26/97	26	5 (4-8)	3 (1-6)	floating habitat, urban area
Lach truong,Thanh Hoa Provin.	VNTHLT	10/25/97	33	13 (9-16)	22 (9-53)	fishery area, aquaculture area
Ron river estuary, Ky Anh Provin.	VNKARR	10/18/97	50	10 (8-13)	21 (9-119)	fishery area
Lang Co, Hue City	VNHULC	10/12/97	143	7 (6-9)	4 (1-8)	remote pristine area
Thi Nai, Binh Dinh Province	VNBDTN	10/05/97	54	7 (6-9)	7 (3-15)	shipping line, aquaculture area
Phan Ri estuary, Phan Ri	VNPRPE	09/25/97	30	7 (5-11)	9 (2-25)	fishery area
CAMBODIA						
Koh Kang-1, Koh Kong	CAKKKK-1	07/21/98	46	71 (59-95)	4 (2-8)	fishery area
Koh Kang-2, Koh Kong	CAKKKK-2	07/21/98	52	68 (43-90)	4 (2-8)	fishery area
Koh Kong-3, Koh Kang	CAKKKK-3	07/21/98	59	66 (22-90)	3 (0.6-7)	fishery area
Lo Tangao-1, Koh Kong	CAKKLT-1	07/21/98	98	53 (35-75)	1 (0.4-3)	fishery area
Lo Tangao-2, Koh Kong	CAKKLT-2	07/21/98	75	58 (37-77)	2 (0.4-6)	fishery area
Tachat, Koh Kong	CAKKTC	07/21/98	75	58 (45-75)	1 (0.5-3)	fishery area
Tomnup Rolork, Sihanouk ville	CASVTR	08/15/98	14	96 (54-96)	5 (2-27)	international and commercial harbor
CHINA						
Kat O Chau, Hong Kong	CHHKKO	10/12/98	18	91 (80-96)	16 (12-18)	aquaculture area
Sha Tau Kok, Hong Kong	CHHKST	03/15/99	20	95 (90-98)	17 (13-19)	aquaculture area
CUMC, Hong Kong	CHHKCU	10/12/98	24	86 (82-95)	13 (11-16)	small harbor
Ma On Shan, Hong Kong	CHHKOS	03/15/99	25	65 (60-74)	7 (5-9)	small harbor
Ma Wan, Hong Kong	CHHKMW	03/15/99	21	97 (92-101)	18 (14-20)	aquaculture area
Tai Tau Chau, Hong Kong	CHHKTT	06/29/99	20	99 (90-114)	17 (13-23)	aquaculture area
Tsim Sha Tsui, Hong Kong	CHHKTS	06/23/99	26	66 (60-78)	8 (5-11)	shipping line, commercial harbor
Shai Wan Ho, Hong Kong	CHHKSW	07/20/98	20	86 (80-94)	13 (11-15)	shipping line, marina
Chaung Chau, Hong Kong	CHHKCC	06/25/99	21	91 (84-96)	14 (12-16)	aquaculture area
Sok Kwu Wan, Hong Kong	CHHKSK	06/23/99	25	78 (72-88)	10 (9-13)	power station, boatyard, aquaculture area
Xiamen, Southern China	CHXMLH	05/15/99	24	74 (68-82)	9 (6-12)	aquaculture area
Shenzhen, Southern China	CHSZWS	05/15/99	18	85 (78-91)	13 (11-14)	small harbor, industrial area
Fuzhou, Southern China	CHFZLJ	07/05/99	20	79 (72-88)	10 (9-13)	aquaculture area
INDIA						
Digha, West Bengal	INWBDH	01/17/98	15	81 (64-104)	18 (6-29)	fishing harbor
Subarnarekha, Orissa	INOSSK	01/12/98	15	111 (88-129)	30 (17-50)	fishing harbor
Gopalpular, Orissa	INOSGL	02/20/98	24	100 (90-107)	24 (9-22)	fishing harbor
Vishakhapatnam, Vizag	INVZVN	02/24/98	10	132 (113-146)	35 (17-73)	small harbor
Kakinada, Andhra Pradesh	INAPKD	02/23/98	14	133 (108-144)	30 (18-63)	aquaculture area
Machilipatnam, Andhra Pradesh	INAPMN	02/22/98	19	107 (96 -124)	18 (13-27)	fishing harbor
Ennore, Tamil Nadu	INTNEN	03/03/98	40	77 (55-102)	9 (3-16)	industrial area
Kasimedu, Tamil Nadu	INTNKD	03/03/98	37	57 (36-69)	5 (3-9)	fishing harbor
Pondicherry, Pondicherry	INPCPC	01/21/98	46	74 (46-129)	20 (3-36)	small harbor
Cuddalore, Tamil Nadu	INTNCD	01/21/98	29	102 (83-128)	15 (9-21)	aquaculture area
Parangipettai, Tamil Nadu	INTNPP	03/04/98	26	69 (46-98)	8 (3-17)	aquaculture area
Nagappattinam, Tamil Nadu	INTNNN	02/02/98	16	91 (78-103)	11 (6-16)	fishing harbor
Akkaraipettai, Tamil Nadu	INTNAN	02/21/98	22	82 (37-105)	9 (1-16)	fishing harbor
Kanniya Kumari, Tamil Nadu	INTNKN	03/02/98	38	97 (87-105)	13 (10-16)	fishing harbor
Cochin, Kerala	INKLCH	03/05/98	27	87 (72-126)	n.a.	harbor, aquaculture area
Goa, Goa	INGOGA	03/19/98	49	71 (86-53)	8 (4-12)	urban area, small harbor
Bombay,Maharashtra	INMHMB	03/19/98	31	73 (62-88)	n.a.	urban area, harbor
INDONESIA						
Belawan, Medan	IDBLMD	07/12/98	48	90 (66-120)	11 (3-19)	commercial harbor
Kuala Tungkal, Jambi	IDJAKT	10/15/98	51	69 (54-86)	5 (3-8)	aquaculture area
T. Hurun, Lampung	IDLATH	07/23/98	40	83 (70-102)	7 (4-13)	aquaculture area
Lada Bay, Panimbang	IDLBPN	08/01/98	56	58 (45-75)	4 (2-7)	aquaculture area
Kamal, Jakarta	IDJKKA	07/29/98	54	78 (60-94)	8 (4-13)	fishing harbor, industrial area
Cilincing, Jakarta	IDJKCL	07/28/98	49	60 (51-70)	5 (4-8)	fishing harbor, industrial area
Ancol, Jakarta	IDJKAC	07/28/98	51	45 (35-65)	3 (2-6)	marina, harbor, industrial area
Bondet, Cirebon	IDBDCB	08/03/98	51	81 (71-94)	9 (5-14)	fishery area
Genjeran, Surabaya	IDGJSB	07/20/98	50	74 (48-91)	8 (3-13)	harbor, industrial area
Maros, Ujung Pandang	IDMRUP	07/16/98	24	105 (95-123)	19 (13-31)	fishery area
MALAYSIA						
Trayong, Sabah	MYSATR	08/29/98	25	70 (64-74)	3 (1-4)	agriculture, aquaculture area
Kuala Penyu, Sabah	MYSAKP	08/29/98	25	80 (74-86)	6 (3-6)	agriculture, aquaculture area
Sangkar Ikan-1, Langkawi	MYKESI-1	12/25/97	25	78 (52-90)	5 (3-6)	urban area, aquaculture area
Sangkar Ikan-2, Langkawi	MYKESI-2	09/20/98	25	70 (62-91)	4 (3-8)	urban area, aquaculture area
Tanjung Rhu, Langkawi	MYKETR	09/20/98	25	71 (67-75)	5 (3-6)	recreational beach, aquaculture area
Tg. Dawai, Kedah	MYKETD	12/26/97	25	89 (81-102)	7 (2-10)	fishery, aquaculture area
Pn. Bridge-1, Penang	MYPEPB-1	03/14/97	25	71 (66-74)	5 (3-8)	harbor, industrial area, urban area
Pn. Bridge-2, Penang	MYPEPB-2	09/21/98	25	73 (48-100)	6 (5-7)	harbor, industrial area, urban area
Bg. Lalang, Selangor	MYSEBL	06/08/98	25	87 (74-97)	6 (3-7)	recreational beach, aquaculture area
Lukut, Negeri Sembilan	MYNSLU	08/08/98	25	86 (71-98)	9 (8-13)	harbor, aquaculture area
P. Panjang-1, Negeri Sembilan	MYNSPP-1	08/19/98	25	92 (78-104)	10 (7-12)	recreational beach, aquaculture area
P. Panjang-3, Negeri Sembilan	MYNSPP-3	09/22/98	25	89 (68-101)	8 (3-10)	recreational beach, aquaculture area
Tanjung Batu, Melaka	MYMAAB	09/22/98	25	92 (84-101)	8 (5-11)	agriculture, aquaculture area
Tg. Kupang, Johore	MYJBTK	07/18/97	25	78 (66-92)	8 (6-10)	harbor, aquaculture area
Pantai Lido-2, Johore Bahru	MYJBPL-2	05/30/98	27	67 (55-81)	6 (3-7)	harbor, sipping line, urban area
Pantai Lido-3, Johore Bahru	MYJBPL-3	09/23/98	25	59 (47-72)	4 (3-7)	harbor, shipping line, urban area
Pasir Putih-2, Johore Bahru	MYJBPP-2	05/30/98	25	73 (68-88)	6 (3-7)	harbor, shipping line, urban area
Pasir Putih-3, Johore Bahru	MYJBPP-3	09/23/98	25	64 (42-100)	6 (4-7)	harbor, shipping line, urban area
PHILIPPINES						
Pamarawan, Bulacan	PHBLPW	03/26/98	15	81 (74-93)	8 (6-107)	coastal (small boating activity)
Obando, Bulacan	PHBLOD	03/17/98	82	58 (17-89)	4 (2-10)	coastal (small boating activity)
Malabon, Metro Manila	PHMMMB	03/16/98	71	63 (41-87)	6 (2-14)	coastal (small boating activity)
Bacoor, Cavite	PHCVBC	03/27/98	43	69 (60-77)	7 (5-19)	coastal (small boating activity)
San Pedro Bay, Leyte	PHLTSP	32198	15	104 (89-117)	17 (13-27)	aquaculture area
Jiabong, Samar	PHSMJB	03/22/98	46	67 (50-95)	5 (2-13)	aquaculture area
Villareal, Samar	PHSMVR	03/23/98	51	67 (51-84)	4 (2-8)	aquaculture area
Sapia Bay, Capiz	PHCZSP	04/08/98	87	64 (41-87)	4 (2-10)	aquaculture area

Figures in parentheses indicate ranges

[a] First, second and third letters indicate the abbreviation of country, province or city and local name, respectively

n: number of individual samples

n.a.: no data available

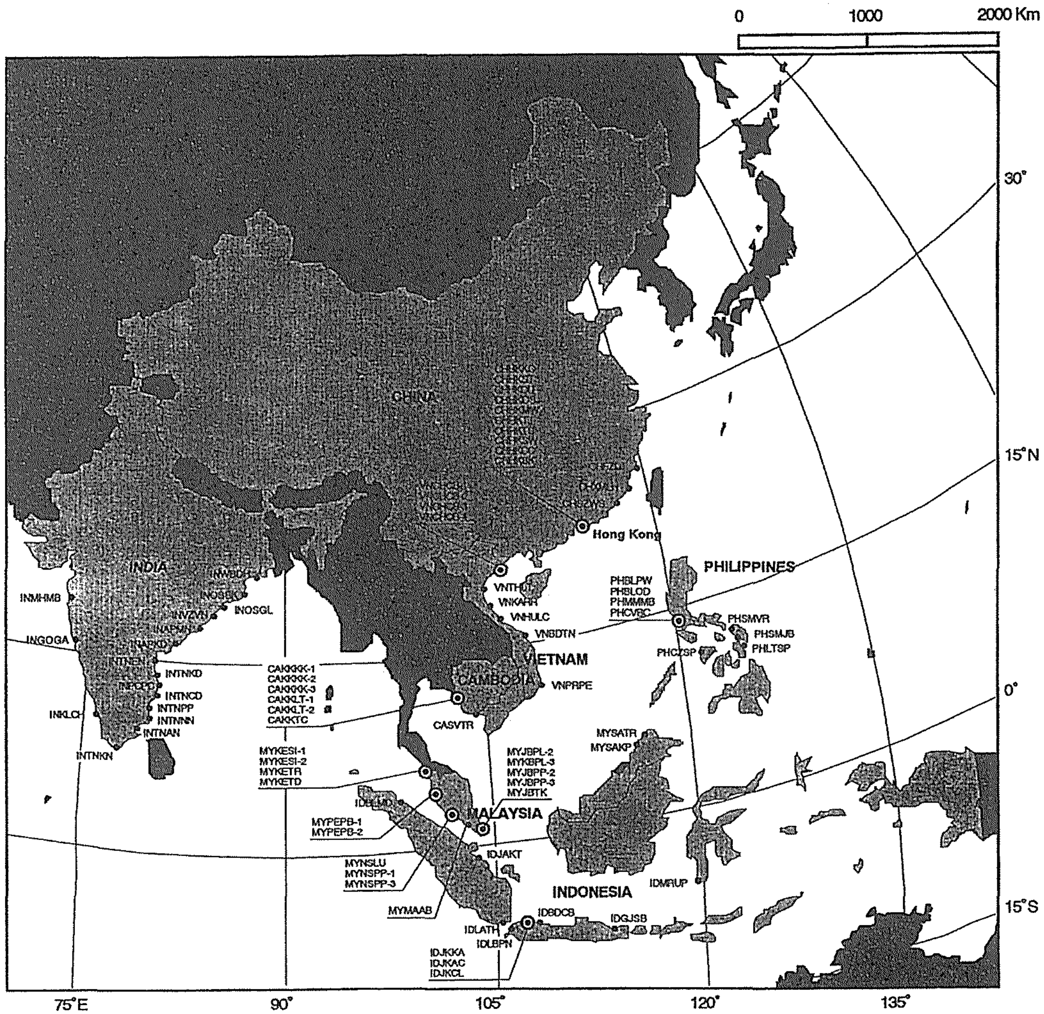

Fig. 1. Map showing sampling locations of green mussels (*Perna viridis*) in coastal waters of Asian developing countries. The site codes of sampling locations refer to Table 1.

Folsom CA, 100% dimethyl polysiloxane) was used for separation. Detection limits of BTs in samples were assigned twice the values of the procedural blanks. The recovery rates through the whole analytical procedure for MBT, DBT and TBT spiked into the liver of Antarctic minke whale were 111 ± 17%, 121 ± 16 % and 92 ± 7.7 %, respectively (n = 5). The concentrations of BTs are reported as nanogram of corresponding ion per gram on a wet weight basis.

The analytical procedure for total tin was based on the method described elsewhere [30, 31] with slight modification. Tin concentrations in samples were determined using inductively coupled plasma mass spectrometry (ICP-MS) (Hewlett Packard, HP 4500). Detection limit was 10 ng Sn/g on a dry weight basis. Accuracy of the analytical method was checked using a certified biological reference material (NIES No. 11) and the recovery of Sn obtained was 107±1.2% (n=3).

Results and Discussion

Contamination status, distribution and pollution sources

Concentrations of BTs detected in mussels from Vietnam, Cambodia, China, India, Indonesia, Malaysia and Philippines are shown in Table 2. BTs were detected in mussels from all the sampling locations investigated. Considering the results of our previous studies in Thailand [27], India [28] and the Philippines [29] with this study, it is evident that butyltin contamination is widespread along coastal waters of many Asian developing countries including Vietnam. Among BTs, TBT was detected at all locations at relatively higher concentrations, whereas the concentrations of DBT and MBT were lower. This indicates that green mussel, similar to other mollusks, has a limited ability to metabolize TBT to DBT and MBT [32, 33]. In addition, this may suggest fresh inputs of TBT into the aquatic environment and the presence of recent sources along the coastal waters of Asian countries.

The concentrations of BTs varied widely depending on the locations. To describe the distribution of butyltin contamination in Asian developing countries, the results obtained in this study were combined and discussed with those reported earlier. Fig. 2 shows distribution of total butyltin concentrations (∑BTs = MBT + DBT +TBT) in mussels from various coastal locations of Asian developing countries investigated in the APMW project. The highest concentration of ∑BTs (960 ng/g wet wt) was found in mussels collected at the narrowest of Johore Strait, Malaysia (MYJBPL-2). Higher concentrations were also observed at the other sites along Malacca Strait, Malaysia. The highly contaminated spots were also found in the coasts of West Bengal and Orissa States, India (INWBDH and INOSSK), where ∑BTs concentrations in mussels were 720 ng/g and 760 ng/g wet wt, respectively. Similarly, considerably high concentrations of ∑BTs were also observed in mussels from several sampling sites in Hong Kong where the concentrations in mussels were up to 500 ng/g wet wt (at CHHKSK). In addition to these locations investigated in this study, several locations along the northern coasts of Gulf of Thailand and Manila Bay, Philippines, which were observed in previous studies [27, 29], appeared to be one of the highly contaminated areas in the Asia-Pacific region (Fig. 2). ∑BT concentrations in green mussels from these locations were reported up to 800 ng/g (at an aquaculture site in Gulf of Thailand) and 790 ng/g wet wt (at one site along Manila Bay, Philippines), respectively. Moreover, a recent study conducted in South Korea using blue mussels (*Mytilus edulis*) [34] showed considerably high contamination along south coast of this country, particularly in the vicinity of shipyards where ∑BTs concentrations in mussels were up to 2500 ng/g wet wt (Fig. 2).

Almost all the locations with high concentrations in mussels have intensive maritime activities, i.e. in the vicinity of large harbors, major shipping traffic areas (e.g. MYJBPL-2 and -3, Malaysia and CHHKTS, Hong Kong), marinas (e.g. CHHKSW, in Hong Kong), boat and shipyards (e.g. CHHKSK, Hong Kong and southern coast of Korea), fishing harbors (e.g. INWBDH and INOSSK, India), international and commercial harbors (Manila Bay, Philippines and northern coasts of Gulf of Thailand). Despite relatively lower contamination, mussels from Cambodia, Indonesia and Vietnam also showed higher concentrations at locations in proximity to international and commercial harbors (e.g. CASVTR), marinas (e.g. IDJKAC), fishing harbors (e.g. IDJKCL) or shipping traffic lines (e.g. VNBDTN). Considering these results, it can be suggested that maritime activities contribute to BT sources in these countries, i.e. ongoing and significant usage of TBT as a biocide in antifouling paints on ship hulls and/or

Table 2. Concentrations of butyltin compounds, total tin (ΣSn) and percentage of TBT in total butyltin (ΣBTs) and total butyltin in total tin in green mussels collected from coastal waters of Vietnam, Cambodia, China, India, Indonesia, Malaysia and Philippines.

Location	MBT	DBT	TBT	ΣBTs[a]	*TBT/ΣBTs*[a]	ΣBTs[a]	ΣSn	*ΣBTs[a]/ΣSn*
	ng/g wet wt				%	ng/g dry wt		%
VIETNAM								
VNCHCB-1	2.5	5.4	16	24	*67*	60	140	*43*
VNCHCB-2	<2.1	4.2	8.4	13	*65*	37	97	*38*
VNCHCB-3	<2.1	6.4	5.5	12	*46*	32	510	*6.2*
VNCHCB-4	2.3	4.3	5.1	12	*43*	32	67	*47*
VNTHLT	3.3	9	15	27	*56*	67	89	*75*
VNKARR	<2.1	3.2	2.9	6.1	*48*	16	23	*70*
VNHULC	<2.1	<0.86	2.1	2.1	*100*	6	110	*5.4*
VNBDTN	<2.1	19	84	100	*84*	280	640	*43*
VNPRPE	<2.1	4	8.5	13	*65*	41	81	*50*
CAMBODIA								
CAKKKK-1	<2.0	1.6	3	4.6	*65*	13	32	*41*
CAKKKK-2	<2.0	<0.98	2.4	2.4	*100*	4	49	*8.2*
CAKKKK-3	<2.0	<0.98	2.5	2.5	*100*	6.4	53	*12*
CAKKLT-1	<2.0	1	2.7	3.7	*73*	12	100	*12*
CAKKLT-2	<2.0	<0.98	2.6	2.6	*100*	6.9	70	*9.8*
CAKKTC	18	1.1	2.5	22	*11*	100	75	*133*
CASVTR	25	37	88	150	*59*	310	380	*82*
CHINA								
CHHKKO	94	33	330	460	*72*	1400	1700	*82*
CHHKST	46	17	160	220	*73*	800	740	*108*
CHHKCU	20	14	37	71	*52*	160	250	*64*
CHHKOS	17	10	22	49	*45*	170	290	*59*
CHHKMW	43	36	210	290	*72*	790	730	*108*
CHHKTT	70	32	94	200	*47*	840	1100	*76*
CHHKTS	46	38	330	410	*80*	1300	1500	*87*
CHHKSW	90	39	320	450	*71*	1700	1700	*100*
CHHKCC	38	23	180	240	*75*	550	640	*86*
CHHKSK	89	76	330	500	*66*	580	430	*135*
CHXMLH	12	10	86	110	*78*	470	420	*112*
CHSZWS	9	4.9	16	30	*53*	93	220	*42*
CHFZLJ	4.2	7.6	20	32	*63*	59	71	*83*
INDIA								
INWBDH	66	88	570	720	*79*	1300	1400	*93*
INOSSK	66	150	540	760	*71*	1400	1400	*100*
INOSGL	15	4.3	0.99	20	*5*	64	100	*64*
INVZVN	<2.2	<0.86	0.85	0.85	*5*	1.9	100	*1.9*
INAPKD	2.5	4.3	1	7.8	*13*	18	96	*19*
INAPMN	10	8.3	18	36	*50*	95	120	*79*
INTNEN	2.5	1	1.5	5	*30*	15	220	*6.8*
INTNKD	15	17	75	110	*68*	190	200	*95*
INPCPC	<2.2	1.4	1.8	3.2	*56*	6.2	67	*9.3*
INTNCD	<2.2	<0.86	1.8	1.8	*100*	3.2	30	*11*
INTNPP	<2.2	0.86	0.83	1.7	*49*	3.9	37	*11*
INTNNN	<2.2	<0.86	3.6	3.6	*100*	8.4	25	*34*
INTNAN	<2.2	<0.86	2.4	2.4	*100*	5.5	63	*8.7*
INTNKN	<2.2	<0.86	1.6	1.6	*100*	3.6	67	*5.4*
INKLCH	<2.2	<0.86	1.9	1.9	*100*	3.3	31	*11*
INGOGA	<2.2	11	43	54	*80*	140	170	*82*
INMHMB	<2.2	4	8.5	13	*68*	31	50	*62*
INDONESIA								
IDBLMD	2.8	<0.58	2.2	5	*44*	15	260	*5.8*
IDJAKT	<1.5	<0.58	3.7	3.7	*100*	9	220	*4.1*
IDLATH	2.6	3	4.3	9.9	*43*	32	180	*18*
IDLBPN	1.5	1.9	3.4	6.8	*50*	17	250	*6.8*
IDJKKA	7.6	6.7	13	27	*48*	88	260	*34*
IDJKAC	13	14	37	64	*58*	150	300	*50*
IDJKCL	11	8.6	38	58	*66*	150	300	*50*
IDBDCB	2.3	2	2.9	7.2	*40*	20	210	*9.5*
IDGJSB	11	6.4	28	45	*62*	120	270	*44*
IDMRUP	2.5	1.1	2.5	6.1	*41*	17	160	*11*
MALAYSIA								
MYSATR	<2.6	<1.0	3.5	3.5	*100*	11	59	*19*
MYSAKP	3.6	<1.0	3.5	7.1	*49*	28	59	*48*
MYKESI-1	17	6	48	71	*68*	190	240	*79*
MYKESI-2	22	12	110	140	*79*	430	550	*78*
MYKETR	6.5	4.1	11	22	*50*	59	130	*45*
MYKETD	5.7	2.4	22	30	*73*	84	260	*32*
MYPEPB-1	31	25	260	320	*81*	710	1500	*47*
MYPEPB-2	58	20	150	230	*65*	690	920	*75*
MYSEBL	13	3.6	26	43	*60*	120	230	*52*
MYNSLU	26	10	89	130	*68*	300	500	*60*
MYNSPP-1	<2.6	1.4	11	12	*92*	26	460	*5.7*
MYNSPP-3	<2.6	2	14	16	*88*	35	250	*14*
MYMAAB	7.7	2.4	15	25	*60*	76	240	*32*
MYJBTK	23	20	88	130	*68*	280	410	*68*
MYJBPL-2	66	160	730	960	*76*	2600	2000	*130*
MYJBPL-3	74	92	350	520	*67*	1200	1800	*67*
MYJBPP-2	13	8.3	50	71	*70*	180	280	*64*
MYJBPP-3	9.3	19	91	120	*76*	260	440	*59*
PHILIPPINES								
PHBLPW	<2.0	2.1	1.7	3.8	*45*	9.6	28	*34*
PHBLOD	7.7	5.8	3.9	17	*23*	48	170	*28*
PHMMMB	8.1	19	47	74	*64*	150	180	*83*
PHCVBC	15	18	31	64	*48*	150	140	*107*
PHLTSP	4.7	2.8	9.8	17	*58*	47	74	*64*
PHSMVR	<2.0	<1.3	0.8	0.8	*100*	1.6	38	*4.2*
PHSMJB	<2.0	<1.3	0.8	0.8	*100*	1.7	17	*10*
PHCZSP	2.1	1.6	3	6.7	*45*	16	59	*27*

[a]ΣBTs = MBT + DBT + TBT

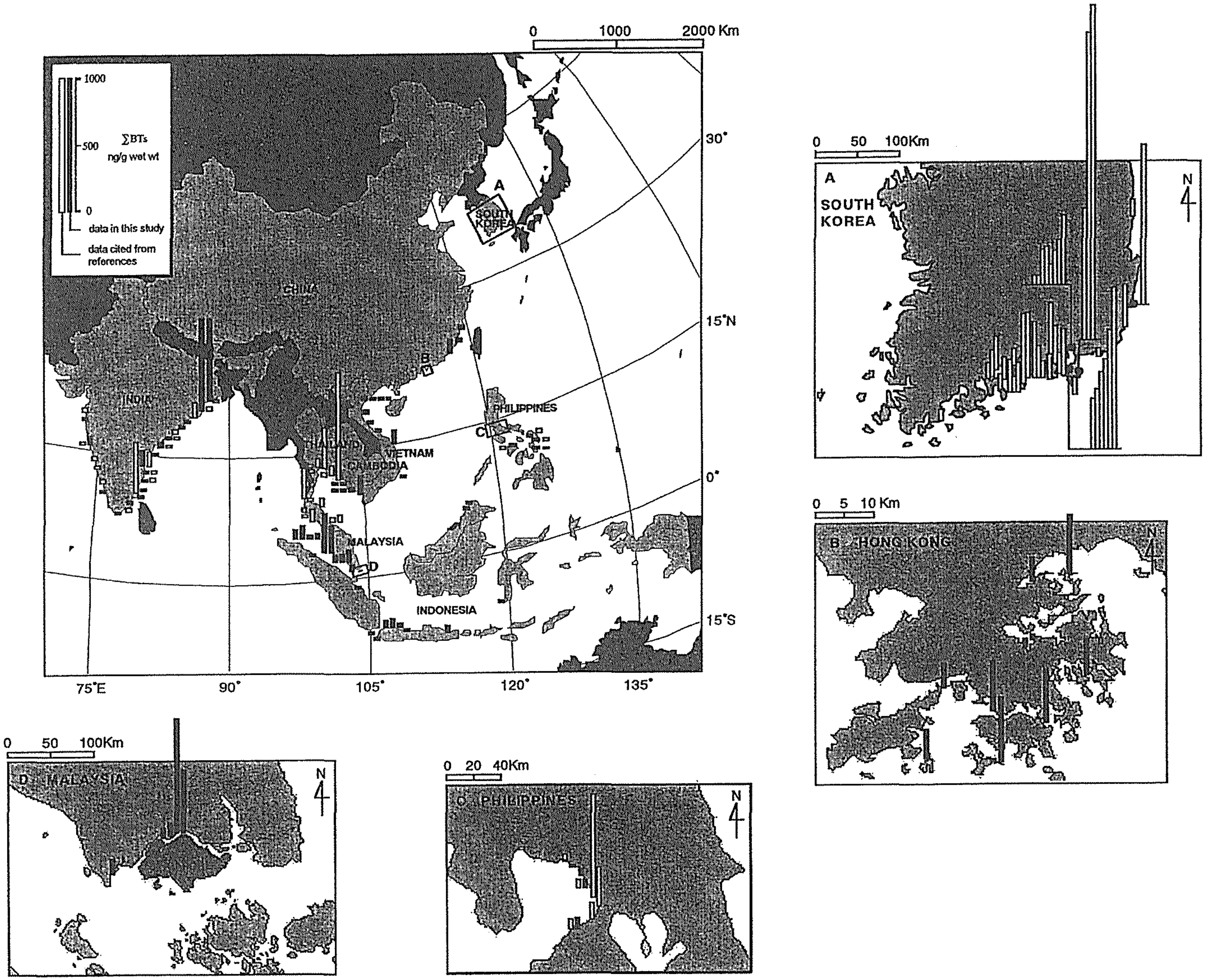

Fig. 2. Distribution of total butyltin concentrations (∑BTs = MBT + DBT + TBT) in mussels collected from coastal waters of some Asian developing countries such as Cambodia, China (Hong Kong and southern China), Malaysia, India, Indonesia, Philippines, Thailand and Vietnam. Data for Thailand, India, Philippines and South Korea include those cited from Kan-atireklap et al. (1997 and 1998), Prudente et al. (1999) and Hong et al. (2001), respectively, which are shown as white bars. Data for South Korea were based on the concentrations in blue mussels (*Mytilus edulis*), but those for all the other countries were based on the concentrations in green mussels (*Perna viridis*).

other marine structures in harbors. Many studies have also documented the existence of significant TBT contamination in harbors, marinas, shipyards and high boating activities in developed nations [13, 35-38]. The results of this study agreed with those reported in other investigations in the developed nations and in some Asian developing countries [15, 17, 39].

Moreover, previous study in Thailand suggested the usage of TBT in fishery activities as high concentrations of TBT (up to 680 ng/g wet wt) were found in mussels from aquaculture areas [27]. TBT usage in aquaculture areas was also suggested in a recent monitoring study in South Korea [34]. In the present study, relatively high concentrations of TBT and $\sum$BTs were also observed in mussels from aquaculture areas in Hong Kong (e.g. CHHKKO, CHHKST, CHHKMW, CHHKTT, CHHKCC) and in Malaysia (e.g. MYKESI-2). Lau (1991) reported high concentrations of TBT in seawater and sediment samples collected in mariculture sites in Sai Kung, Hong Kong. These results suggest the presence of significant pollution sources of BTs in aquaculture activities of some Asian countries such as South Korea, Thailand, Hong Kong and Malaysia. However, mussels from aquaculture areas in other Asian developing countries contained lower concentrations of BTs.

Higher concentrations of TBT than DBT and MBT in mussels from almost all the locations in the Asia-Pacific region suggest recent usage of TBT in maritime and/or aquaculture activities, while mussels from some rural areas (e.g. coastal, fishery, aquaculture and remote pristine areas) in Cambodia, India, Indonesia, Philippines and Vietnam contained TBT at lower concentrations and proportions (Table 2), indicating that TBT usage as antifouling agents in these rural areas seems to be minimal. A possible pollution source of BTs in such location may be the usage of MBT and DBT in plastic products as stabilizers and catalysts. In fact, plastic have often been found along the coasts of Asian developing countries, even in the rural sites.

International comparison

To compare the magnitude of contamination among Asian developing countries, Hong Kong and South Korea, range, mean and 90th percentile values of concentrations of TBT and $\sum$BTs in mussels were calculated for each country (Table 3). Mussel samples from Hong Kong, South Korea, Malaysia and Thailand showed relatively high levels of BTs compared with those from other Asian developing countries, whereas the lowest values were observed in those from Vietnam, Cambodia and Indonesia. Interestingly, the orders of mean and 90th percentile values of concentrations agreed well with the per-capita Gross National Product (GNP) of each country (Table 3). Since per-capita GNP is an indicator of economic status, butyltin contamination seems to be strongly related to the industrial and human activities. The contamination of BTs may increase in those countries with high economic growth rate. In addition, remarkable butyltin contamination in South Korea may reflect high ship-building and repairing activities in this country [41]. Moreover, as mentioned above, mussels from aquaculture areas in Hong Kong, South Korea, Malaysia and Thailand contained higher concentrations of BTs. This may indicate that some Asian countries having better economic

Table 3. Range, mean and 90th percentile values of TBT and ΣBTs concentrations in mussels from Asian developing countries, Hong Kong and South Korea

Country	Sampling year	TBT (ng/g wet wt)			ΣBTs[a] (ng/g wet wt)			Per-capita GNP ($)[b]
		Range	Mean	90th percentile	Range	Mean	90th percentile	
Hong Kong	1998-1999	22-330	200	330	49-500	290	480	24716
South Korea[c]	1997-1999	17-1200	230	480	49-2500	530	1100	9511
Malaysia	1997-1998	3.5-730	120	330	3.5-960	160	480	3092
Thailand[d]	1994-1995	3-680	81	200	4-800	100	260	1850
Philippines[e]	1994/1997-1998	<1-640	58	130	n.d.-790	78	180	907
Southern China	1999	16-86	41	n.a.	30-110	60	n.a.	768
India[f]	1994-1995/1998	<1-570	44	84	0.85-760	73	170	436
Indonesia	1998	2.2-38	14	37	3.7-64	23	61	460
Vietnam	1997	2.1-84	16	57	2.1-100	23	71	310
Cambodia	1998	2.4-88	15	71	2.4-150	27	120	270

Figures in parentheses indicate the order of high concentration value

n.d.: not detected

n.a.: no data available

[a] ΣBTs = MBT + DBT + TBT

[b] Data for 1999 cited from Asiaweek (2001)

[c] Data cited from Hong et al. (2001)

[d] Data cited from Kan-atireklap et al. (1997)

[e] Data of this study pooled from Prudente et al. (1999)

[f] Data of this study pooled from Kan-atireklap et al. (1998)

status tend to increase the usage of TBT for aquaculture activities, in addition to boating and shipping activities.

When comparing the data reported for bivalves from various locations of the world, contamination levels of BTs were generally low in mussel samples from most of the Asian developing countries (Table 4). However, some mussel samples from polluted areas in Hong Kong, India, Malaysia, Philippines and Thailand revealed levels comparable to those reported in developed nations including Japan, European and North American countries. In addition, blue mussels from south and east coasts of Korea showed the highest concentrations (Table 4). This indicates that coastal waters of certain regions in Asian developing countries have been affected seriously by butyltin pollution. Considering high economic growth rate, unregulated usage of TBT in most Asian developing countries and increasing demand of marine coating paints in Asia and Oceania [23], contamination by BTs in Asian aquatic environment may become more serious in future. Thus, continuous monitoring and investigations on butyltin contamination are required in Asian developing countries. Although a reduction of TBT contamination after the regulation was recorded in many developed countries including Japan [42], contamination status of BTs in Hong Kong did not seem to improve during 1989 to 1999 (Table 4). This implies continuous pollution by BTs derived from heavy ship traffic, which may come from the countries having no TBT usage regulation, such as in the busiest ports of Hong Kong. No declining trend of the contamination could be suggested in India (comparing with the data between 1994-95 and 1998) and South Korea (between 1994 and 1997-99) (Table 4).

Ecotoxicological perspectives and risk assessment on human health

As an evidence of ecotoxicological impact of TBT, "imposex phenomenon" has been recorded in gastropods in coastal waters of some Asian countries [11, 44-49]. Particularly, high incidences (almost 100%) of imposex in gastropods have been noted in Japanese coastal waters [11, 49], south coasts of Korea [48], Gulf of Thailand and Malacca Strait, Malaysia [44]. This trend agreed with the results of our monitoring survey and other studies in these

Table 4. Range concentrations (ng ion/g wet wt*) of MBT, DBT and TBT residues in bivalves collected from various regions

Species	Location (Year surveyed)	MBT	DBT	TBT	Reference
Mytilus edulis	Pacific Coast, USA (1986-187)	n.a.	n.a.	n.d.-1080	[62]
	East Coast, USA (1989-1990)	nd-28	2-116	2-240	[63]
	West Coast, USA (1989-1990)	nd-60	2-148	2-276	[63]
	Tokyo Bay, Japan (1989)	·20-120	40-450	20-240	[64]
	British Columbia, Canada (1990)	2.4-15	2.7-31	25-153	[65]
	Perth, Australia (1991)	n.a.	n.a.	<1-330	[66]
	Chinhae Bay, Korea (1994)	n.a.	n.a.	59-590	[20]
	Coastal harbor, Canada (1995)	nd-210	nd-416	10-585	[67]
	Otsuchi Bay, Japan (1996)	n.a.	n.a.	28-301	[68]
	Yamada bay, Japan (1996)	n.a.	n.a.	310-1100	[68]
	Osaka Port, Japan (1996)	n.a.	n.a.	24-390	[69]
	South and East Coasts, Korea (1997-1999)	9.3-300	19-1100	17-1200	[34]
Mytilus galloprovincialis	West Coast, Portugal (1986)	3.2-169	n.d.-82	n.d.-114	[70]
	Mediterranian (1988)	n.a.	2.4-490	44-520	[71]
	Western Mediterranean (1996)	10-204	4-1094	1-1151	[72]
Mya areanaria	Denmark (1989)	n.a.	n.a.	250-1470	[73]
Crassostrea virginica	Gulf of Mexico (1989-1991)	1.5-43	2.0-149	n.d.-708	[74]
	East Coast, USA (1989-1990)	n.a.-66	2-314	2-806	[63]
	Cheaspeake Bay, USA (1987)	n.a.	n.a.	<10-5600	[75]
Crassostrea gigas	Coastal estuaries, UK (1986-1989)	n.a.	n.a.	80-6350	[76]
	Western coast, Taiwan (1996-1997)	n.d.-22	5.9-48	20-737	[21]
	Chinhae Bay, Korea (1995)	n.a.	n.a.	232-2159	[19]
Saccostrea commercialis	Hawkesbury River Estuary, Australia (1991)	n.a.	n.a.	nd-1700	[77]
Perna viridis	Hong Kong (1989)	n.a.	n.a.	64-115	[15]
	Thailand (1994-1995)	<3-45	<2-80	3-680	[27]
	Malaysia (1992)	n.a.	n.a.	14.2-23.5	[18]
	India (1994-1995)	<3.0-250	<1.0-110	<1.0-150	[28]
	Philippines (1994-1997)	<3-51	<1-100	<1-640	[29]
	India (1998)	<2.2-66	<0.86-150	0.83-570	This study
	Philippines (1997-1998)	<2.0-15	<1.3-19	0.80-47	This study
	Indonesia (1998)	1.5-13	<0.58-14	2.2-38	This study
	Malaysia (1998)	<2.6-74	<1.0-160	3.5-730	This study
	Cambodia (1998)	<2.0-25	<0.98-37	2.4-88	This study
	Vietnam (1998)	<2.1-3.3	<0.86-19	2.1-64	This study
	Hong Kong (1999)	4.2-90	4.9-76	16-330	This study

* Concentrations expressed as dry weight and Sn were normalized to wet weight and cation of BTs species (wet wt basis conc.= 0.2 x dry wt basis conc.; TBT ion conc. = 2.44 x Sn conc.; DBT ion conc. = 1.96 x Sn conc.; and MBT ion conc. = 1.48 x Sn conc.) in order to compare with this study.
n.d.: not detected
n.a.: no data available

countries; the locations where high incidence of imposex observed are identical with those found to be 'highly' contaminated by BTs (Fig. 2 and Table 4). In addtion, Page and Widdows [50] reported that the threshold for effects on the 'scope for growth' of mussels by TBT was approximately 2 μg/g dry wt (≈ 400 ng/g wet wt). The concentrations of TBT found in green mussels collected from several locations of Asian developing countries were higher than this value. These observations suggest that TBT levels in some polluted areas of Asian developing countries impose toxic threat to susceptible mollusks and other marine organisms.

Butyltin contaminated seafood may cause adverse effects on human health. Mussels are one of the commonly consumed seafood items in Asia-Pacific region [25, 34]. Penninks [51] derived a tolerable daily intake (TDI) of TBT of 0.25 μg/kg/ body weight/day, based on the observed effects of TBT on the immune function in rats with a safety factor of 100 to extrapolate the toxicity test from rats to humans. Belfroid et al. [52] suggested tolerable average residue levels (TARLs) for TBT (and also *indicative* TARLs for DBT, which is assumed to have comparable effect levels for TBT) in seafood products to assess risk to human health. TARLs are calculated as;

$$\text{TARL} = \frac{(\text{TDI} \times 60\ \text{kg bw})}{\text{average daily seafood consumption}}$$

Based on the data of average seafood consumption [53], TARLs for seafood in Cambodia, Hong Kong, India (coastal region), Indonesia, South Korea, Malaysia, Philippines and

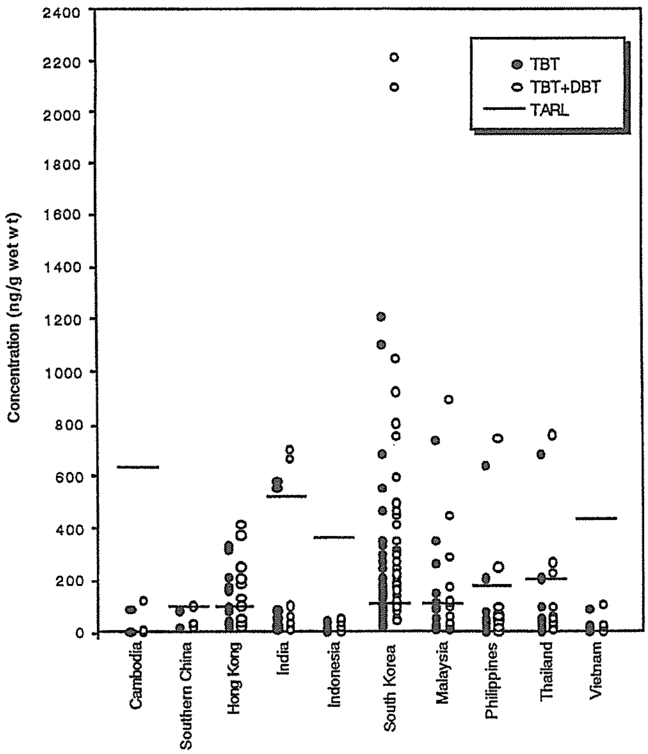

Fig. 3. Residue levels of TBT and sum of TBT and DBT (TBT + DBT) detected in mussels from coastal waters of some Asian countries compared with the tolerable average residue levels (TARLs) for seafood in each country. Data for Thailand, India, Philippines and South Korea include those cited from Kan-atireklap et al. (1997 and 1998), Prudente et al. (1999) and Hong et al. (2001), respectively. Data for South Korea are based on the concentrations in blue mussels (*Mytilus edulis*), but those for all the other countries were based on the concentrations in green mussels (*Perna viridis*). The concept and calculation method of TARLs used here followed that of Belfroid et al. (2000) with slight modification for the average seafood consumption among Indian and Chinese living in the coastal regions (see text).

Vietnam were estimated to be 630, 92, 519, 360, 109, 109, 173 and 435 ng/g for an average person weighing 60 kg, respectively. TARL for seafood in southern China was assumed to be of the same value in Hong Kong. Comparing TARLs in each country, concentrations of TBT or sum of TBT and DBT (TBT +DBT) exceeded those values in some of mussels from southern China, Hong Kong, South Korea, Malaysia, India, Philippines and Thailand (Fig. 3). This result suggests that some Asian people consuming seafood from areas with high butyltin contamination may be at risk due to elevated exposure. In the case of Vietnamese as well as Cambodian and Indonesian, lower risk can be suggested because lower values of BT concentrations than TARLs were observed in mussels from these countries.

Anthropogenic sources

In order to evaluate the extent of anthropogenic input of tin, total tin ($\sum$Sn: organic + inorganic) was determined in mussel samples from Asian countries and the results are compared with the BTs data (Table 2). The highest $\sum$Sn concentration (2000 ng/g dry wt) was found in mussels showing the highest concentration of BTs, which were collected from the narrowest of Johore Strait, Malaysia (MYJBPL-2). The concentrations of $\sum$Sn were generally related to those of BTs; higher concentrations of $\sum$Sn were found in locations, which were considered as areas of great BTs contamination (Fig. 2). Furthermore, a significant positive correlation was observed between the concentrations of $\sum$BTs and $\sum$Sn ($R^2 = 0.85$, $p<0.0001$) in mussel samples (Fig. 4). In fact, $\sum$BTs made up 100% of $\sum$Sn in mussels from locations where intensive maritime and human activities were observed (Tables 1 and 2) (Estimated percentages of $\sum$BTs in Table 2 were somewhat higher than 100 % in some of samples, which could be due to analytical variations in both BTs and $\sum$Sn analysis. It may also be due to differences in recoveries and sample forms employed between these two analyses).

These observations indicate that anthropogenic sources play a major role in tin accumulation in mussels. Particularly in polluted areas, most of the total tin in mussels exists in organic form such as BTs, implying that tin compounds mostly originated from anthropogenic sources. A similar conclusion was obtained in our previous studies with higher trophic marine mammals from coastal waters around Japan and some Asian countries [31, 55]. A study which analyzed organotins such as butyltins and methyltins, and total tin in several fish species, mussels and algae also noted that BTs made up a higher percentage (60% - 90%) of the total tin [56]. Collectively, BTs contribute to significant input of anthropogenic tins in coastal ecosystem and/or indicate high bioaccumulation among aquatic organisms. This feature of BTs is likely to agree with the accumulation of mercury, which is mainly retained as organic form in aquatic organisms [57, 58].

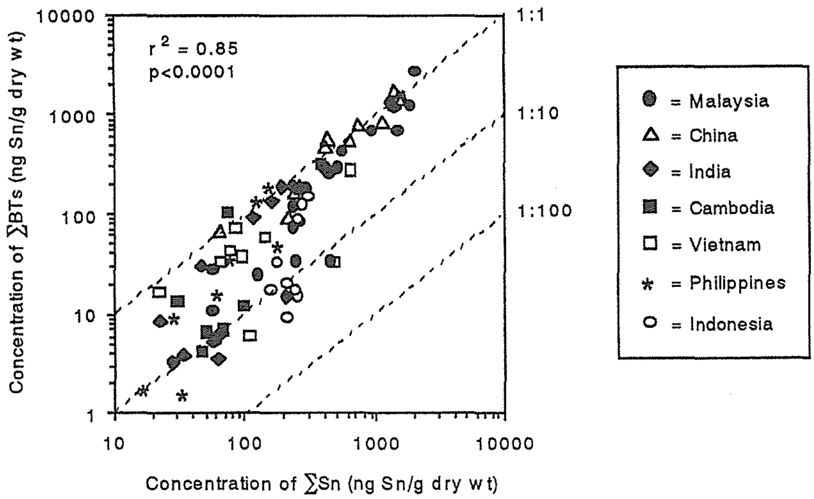

Fig. 4. Relationship between the concentrations of $\sum$BTs and $\sum$Sn in green mussels from the coastal waters of some Asian developing countries.

Although BTs were the major components of tin compounds in mussels from polluted locations, relatively lower proportions of $\sum$BTs in $\sum$Sn were noted in some samples (Table 2 and Fig. 4). This result suggests exposure to other organic and inorganic tin compounds in mussels. It has been documented that phenyl-, octyl- and methyltin compounds were found in some aquatic ecosystems [13, 56, 59, 60]. The pollution of these compounds have originated from many anthropogenic sources due to the compounds being used as stabilizers for polyvinyl chloride (dimethyl- and dioctyltin), fungicides (triphenyltin) and antifoulings (triphenyltin) [13]. Exposure to inorganic tin sources can not be ruled out, because Southeast Asia including Malaysia, Thailand and Indonesia have extensive tin deposit [61].

Conclusions

Results of investigations conducted under the "Asia-Pacific Mussel Watch" clearly indicated widespread butyltin contamination and presence of recent butyltin pollution sources in Asian coastal waters including Vietnam. In the International Maritime Organization (IMO) Assembly conducted in November 1999, an approved resolution prepared by Marine Environmental Protection Committee (MEPC) proposed a global prohibition on the application of organotin compounds as biocides in anti-fouling systems on ships by 1 January 2003, and a complete prohibition on the presence of organotin compounds by 1 January 2008 [43]. The IMO also called for a diplomatic conference in 2001 to consider adoption of the international legal instrument. It is deemed important that Asian developing countries as well as developed nations make agreement for the instrument and ratify an international treaty for the prohibition of organotin compounds usage on a global basis. Moreover, it is necessary to conduct continuous monitoring including the studies in view of ecotoxicological risk assessment to further elucidate the trend of contamination and impacts on organisms in Asian developing countries. Based on present results, the present group of scientists involved in the APMW project agreed to continue to collaborate in conducting continuous monitoring studies and to ensure capacity building and development of highly trained scientists in the countries within the Asia-Pacific region.

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