Research

Butyltin Contamination in Marine Mammals from North Pacific and Asian Coastal Waters

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Hepatic butyltin concentrations were determined in 63 cetaceans belonging to 14 species and four pinnipeds belonging to two species collected from North Pacific and Asian coastal waters. Butyltin compounds (BTs) including tributyltin (TBT), dibutyltin (DBT), and monobutyltin (MBT) were detected in almost all the liver samples suggestive of its worldwide distribution. The elevated residues detected in coastal species and low concentrations found in off-shore species indicate a high degree of butyltin contamination in coastal waters than in the open sea. Mammals inhabiting waters of developed nations were found to contain higher BT concentrations compared with those collected from the waters proximal to developing countries. These observations strongly suggest serious BT contamination in the waters of developed countries than in developing nations at present. Among the samples collected off Japanese coastal waters, lower BT concentrations were found in pinnipeds compared with the cetaceans, suggestive of a possible difference in degradation capacities and excretory moulting between these two groups of animals. The estimated concentration ratio of BT in the liver of killer whale fetus to its pregnant mother was relatively low (0.015), indicative that transplacental transfer of BTs from the mother to her fetus is a deal less. Among the BT breakdown products, DBT was predominant in most of the liver samples analyzed, followed by TBT and MBT.

Introduction

Since the 1960s, butyltin compounds (BTs) have been used worldwide for various purposes; tributyltin (TBT) as antifouling agents in paints used for boats and aquaculture nets and monobutyltin (MBT) and dibutyltin (DBT) as stabilizers for chlorinated polymers or as catalysts for silicones and polyurethane foams (*1*). Widespread usage of these compounds motivated the conduct of numerous studies in order to elucidate environmental contamination and impacts. Earlier studies on BT effects focused mainly on lower trophic organisms in the aquatic food chain. These studies reported physiological abnormalities such as growth reduction in marine microalgae (*2*), shell thickening and spat failure in oysters (*3*, *4*), and imposex in gastropods (*5*) and whelks (*6*, *7*). In view of these investigations, control measures on the usage of BTs were adopted in several countries. However such an action did not appreciably reduce the consumption of organotins on a global scale (*8*). Environmental monitoring and toxicological studies dealing with water (*9*−*11*), sediment (*12*−*14*), mussels (*15*, *16*) and fish (*8*, *17*, *18*) imply that these compounds continue to pose a major ecotoxicological threat in the aquatic environment.

For a comprehensive understanding of BT contamination and their toxic effects in marine ecosystem, our research group conducted studies on BT residues in marine mammals (*19*, *20*). Results of these studies underscored significant accumulation and body distribution of BTs in these higher trophic animals and their appropriateness as bioindicators of aquatic organotin pollution was disclosed. Further investigations on BTs in cetaceans and pinnipeds (*21*−*23*) and fish-eating water birds (*24*−*26*) conducted by our team affirmed the ubiquitous pollution by BTs. Moreover, BT accumulation in other marine vertebrate predators such as marine turtle, bluefin tuna, and shark suggested greater accumulation of these chemicals (*26*, *27*).

Studies on the specific accumulation and distribution of BTs in various organs and tissues of porpoises (*20*) and sea lions (*22*) revealed that BTs accumulated in the liver, suggesting principally its suitability for monitoring studies in aquatic organisms (*8*). This prompted us to analyze BT concentrations in the liver of marine mammals collected from various regions to determine BT contamination levels on a global scale.

In the present study, hepatic concentrations of MBT, DBT, and TBT in marine mammals collected from North Pacific and Asian coastal waters are discussed, and an attempt is made to present global distribution of BTs contamination in marine mammals by comparing the values obtained in our earlier studies on marine mammals, which employed similar analytical methods. Moreover, in order to make clear the transfer mechanism of BTs in these animals, concentration ratio in the liver of a pregnant killer whale and its fetus was calculated.

Materials and Methods

The marine mammals employed for this study were collected from 1979 to 1996 from different locations (Figure 1). Biometric and sampling data of these animals are summarized in Table 1. Almost all of the samples were sexually matured. After dissection, liver was stored in polyethylene bags at −20 °C until analysis.

The chemical analysis of BTs was conducted following the method previously described (*19*). Briefly, about 1−2 g of liver tissue was homogenized with 1 N HCl and 0.1% tropolone/acetone. The BTs in extracts were transferred to 0.1% tropolone/benzene, and the moisture in the solvent was removed with anhydrous Na_2SO_4. BTs in benzene were propylated by adding propyl magnesium bromide as a

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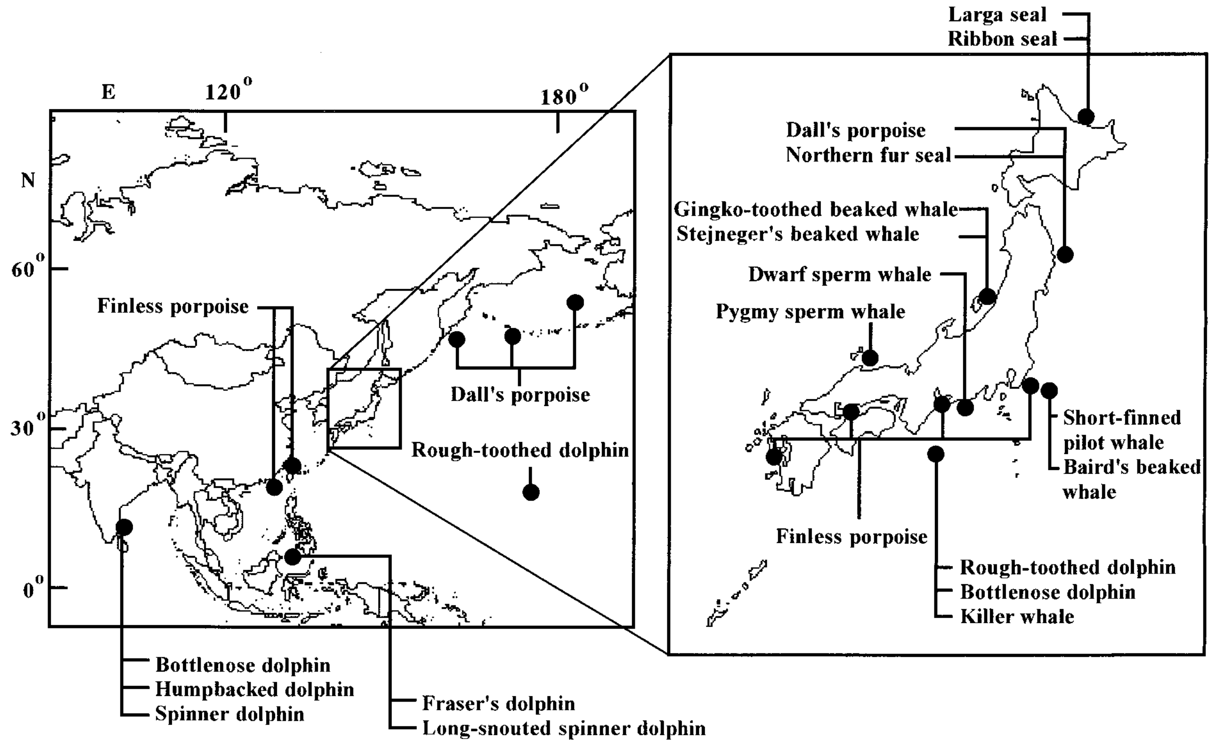

FIGURE 1. Sampling location of marine mammals.

Grignard reagent. The extract was added on a dry florisil column and passed with nitrogen gas slowly. BTs adsorbed on the florisil were eluted with 20% water/acetonitrile to remove lipid. The eluate was then subjected to a wet florisil column for further purification. The final extract was injected into a gas chromatograph with flame photometric detector and a tin mode filter (610 nm). A fused silica capillary column (DB-1; 0.25 mm i.d. × 30 m length) was used for separation. Identification of BTs was made by assigning peaks in samples to corresponding peaks of external standard. Peak heights of individual BTs were used for quantification. Standard mixtures were prepared with every set of four samples by propylating the known amounts of BT ion mixtures spiked on an Antartic minke whale liver, which was previously found to contain trace levels of BTs. Hexyl-tributyltin was added as an internal standard.

In order to examine the recoveries of BTs through the analytical procedure in liver, 0.1 μg of butyltin chloride species dissolved in hexane was spiked into about 2 g of the liver of a whale and subjected to the analysis. Concurrently, butyltin chloride mixture without a matrix was also prepared for reference. Recoveries for MBT, DBT, and TBT in liver matrix were 96.9 ± 26.1, 102 ± 8.7, and 91.1 ± 13.3% ($n = 3$), respectively. In addition, hexyl-tributyltin was also added into the liver, and the recovery was 104 ± 12.8% ($n = 3$). Detection limit was defined as three times the signal-to-noise ratio (4.0 ng g^{-1} for MBT, 1.5 ng g^{-1} for DBT, and 1.0 ng g^{-1} for TBT). The butyltin standards, monobutyltin trichloride, dibutyltin dichloride, and tributyltin chloride were obtained from Kishida Kagaku Co. Ltd. (Osaka, Japan) and Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan) and were of >95% purity. Considering the result of the spiking experiment, reported concentrations of BTs in the liver samples were not corrected for recovery and given as nanograms of butyltin ion per gram on a wet weight basis.

Results and Discussion

BTs were detected in almost all the liver samples (Table 2). Further, no difference was observed in BT concentrations between male and female species, which is similar to that reported for Risso's dolphin (*21*). Due to the smaller number of sample size, age-dependent accumulation was not made clear in the present study. However, in our previous study on Rissos' dolphin (*21*), BT residue levels in adult animals were found to be constant. Similar trend may be expected in other species of cetaceans. Similarly, as the cetaceans analyzed here were mostly adult animals, the geographical variation of BTs levels was considered.

The highest level (10 000 ng g^{-1}) of hepatic ΣBTs (sum of TBT, DBT, and MBT) was found in finless porpoise from the Seto Inland Sea, Japan. Although high accumulation of BTs in porpoises might be partly due to the low cytochrome P450 enzyme activities, as suggested in an earlier study (*20*), the significant hepatic BT residues indicate high degree of BT contamination in the coastal waters of Japan, attributed mainly to heavy maritime activities in the area. It has been reported that TBT is still being used as an antifouling agent for specific shipping vessels in Japan (*20*), thus continuing inputs of this toxic substance are more likely to be occurring.

In this study, cetaceans from other coastal regions around Japan, likewise, revealed elevated hepatic BTs ranging from 110 to 5200 ng g^{-1} wet wt. The BT concentrations in cetaceans collected from Japanese coastal waters were comparable to those in two stranded dolphins found along Italian coastal waters (*27*) and along U.S. Atlantic and Gulf coasts (*23*), but higher than those in drowned porpoises collected from Turkish coast (*28*) (Figure 2). High BT contamination in animals from Japanese coasts might suggest intensive usage of organotins for shipping and aquaculture activities similar to that in other developed nations. Among cetaceans collected from Japanese coastal waters, off-shore species such as Dall's porpoises, beaked whales, and sperm whales were found to contain lower hepatic BTs residues (Table 2), suggesting their minimal exposure to this compound in coastal waters. The BT level measured in a pygmy sperm whale collected off Tottori, Japan, was found to contain similar concentrations measured in sperm whales found stranded in the U.S. coasts (*23*). Further, lower contamination of BTs in off-shore species than in coastal species observed in the present study is in accordance with the results

TABLE 1. Marine Mammals Employed for the Analysis[a]

country/region	species	location	year	WBL (cm)	N M	N F
Northwest Pacific	Dall's porpoise *Phocaenoides dalli*	Aleutian Chain	1979	182	1	0
		Bering Sea	1981	204−213	2	0
		northwestern N. Pacific	1984		3	0
Japan	cetaceans					
	Dall's porpoise *Phocaenoides dalli*	Sanriku Coast	1995	163−195	3	0
	Stejneger's beaked whale *Mesoplodon stejnegeri*	Niigata	1993	429	1	0
	ginkgo-toothed beaked whale *Mesoplodon ginkgodens*	Yamagata	1993	479	1	0
	Baird's beaked whale *Berardius bairdii*	Ayukawa	1988	930−1060	2	1
	short-finned pilot whale *Globicephala macrorhyncus*	Ayukawa	1985	355−658	2	4
			1986	369	1	0
	bottlenose dolphin *Tursiops truncatus*	Taiji	1986	254−287	3	1
	rough-toothed dolphin *Steno bredanensis*	Taiji	1986	169	1	0
	killer whale *Orcinus orca*	Taiji	1986	598−636	1	2
	dwarf sperm whale *Kogia simus*	Toyohashi	1993	265	1	0
	pygmy sperm whale *Kogia briveceps*	Tottori	1997	270	1	0
	finless porpoise *Neophocaena phocaenoides*	Chiba, Pacific Coast	1981	151	1	0
		Seto Inland Sea	1985	162	1	0
		Nagasaki	1992	116	1	0
		Ise Bay	1994	139	1	0
	pinnipeds					
	larga seal *Phoca largha*	Rausu, Hokkaido Coast	1995	180	1	0
	ribbon seal *Histriophoca faciata*	Rausu, Hokkaido Coast	1995	118	1	0
	northern fur seal *Callorinus ursinus*	Sanriku Coast	1990	127	1	0
China	finless porpoise *Neophocaena phocaenoides*	Dongshan	1990−1991	146−168	4	1
		Lusi	1991	122−156	1	2
Philippines	long-snouted spinner dolphin *Stenella longirostris*	Sulu Sea	1996	182−192	1	1
	Fraser's dolphin *Lagenodelphis hosei*	Sulu Sea	1996	221−225	1	1
West Pacific	rough-toothed dolphin *Steno bredanensis*	Pacific Ocean	1983	205−216	5	0
India	Indo-Pacific hump-backed dolphin *Sousa chinensis*	Bay of Bengal	1988−1992	194−225	2	2
	long-snouted spinner dolphin *Stenella longirostris*	Bay of Bengal	1988−1992	117−170	2	2
	bottlenose dolphin *Tursiops truncatus*	Bay of Bengal	1988−1992	169−230	1	3

[a] *N*, sample number; M, male; F, female; WBL, whole body length.

of previous studies (*23, 26*). Nevertheless, in spite of much lower BT levels observed in open-ocean species, it seems clear that BT pollution has extended worldwide.

Relatively low concentrations of BTs were measured in the liver of cetaceans from the coastal waters of the Philippines and India, which ranged 42−98 and 53−200 ng g^{-1} wet wt, respectively. Cetaceans collected from the coast of China revealed relatively higher concentrations ranging from 350 to 1200 ng g^{-1} wet wt. However, cetaceans inhabiting waters adjacent to developing countries in the tropics and subtropics, such as India, Philippines, and China, contain significantly lower hepatic BT concentrations ($p <$ 0.001, Mann-Whitney *U*-test) compared with those inhabiting temperate waters proximal to developed nations, such as Japan, U.S.A., and Italy (Figure 2). This could be indicative of significant and continuous inputs of BTs in the coastal waters of these developed countries, while smaller usage in developing countries is implied at least at the present. An earlier study using fish samples from developing countries of Asia also revealed lower concentrations of BTs (*8*). However, future loading of BTs is expected to increase in view of the increasing demand for paints in the Asia-Pacific (*29*).

Low levels (41−180 ng g^{-1} wet wt) were detected in Dall's porpoises collected from the northwest North Pacific, and even lower concentrations (17−37 ng g^{-1} wet wt) were found in rough-toothed dolphins from the western Pacific Ocean. The remoteness of the sampling site to possible sources of BTs could account for the low levels. However, it should be noted that these cetaceans were collected in late 1970s and early 1980s, thus, the measured residues could be reflective of contamination during that period when usage of organotin

TABLE 2. Butyltin Concentrations (ng/g, wet wt) in the Liver of Marine Mammals from North Pacific and Asian Coastal Waters[a]

country/region	species	location	MBT	DBT	TBT	ΣBTs
Northwest Pacific	Dall's porpoise *Phocaenoides dalli*	Aleutian Chain	**33**	**59**	**26**	**120**
		Bering Sea	**27** (22−32)	**42** (29−55)	**16** (12−19)	**85** (63−110)
		northwestern N. Pacific	**37** (17−58)	**44** (15−93)	**16** (12−19)	**97** (41−180)
Japan	cetaceans					
	Dall's porpoise *Phocaenoides dalli*	Sanriku Coast	**97** (50−120)	**430** (180−600)	**230** (110−310)	**760** (340−1000)
	Stejneger's beaked whale *Mesoplodon stejnegeri*	Niigata	**67**	**280**	**52**	**400**
	ginkgo-toothed beaked whale *Mesoplodon ginkgodens*	Yamagata	**120**[b]	**130**[b]	**76**[b]	**330**[b]
	Baird's beaked whale *Berardius bairdii*	Ayukawa	**46** (17−95)	**140** (80−180)	**23** (9−30)	**210** (110−310)
	short-finned pilot whale *Globicephala macrorhyncus*	Ayukawa	**510** (340−650)	**1200** (770−1600)	**350** (260−520)	**2100** (1500−2600)
		Taiji	**350**	**660**	**280**	**1300**
	bottlenose dolphin *Tursiops truncatus*	Taiji	**480** (310−560)	**1900** (1600−2100)	**470** (390−540)	**2800** (2600−3000)
	rough-toothed dolphin *Steno bredanensis*	Taiji	**450**	**2200**	**670**	**3300**
	killer whale *Orcinus orca*	Taiji	**710** (480−1100)	**1600** (1500−1900)	**180** (150−220)	**2500** (2200−2700)
	dwarf sperm whale *Kogia simus*	Toyohashi	**200**	**470**	**55**	**730**
	pygmy sperm whale *Kogia briveceps*	Tottori	**75**	**120**	**31**	**230**
	finless porpoise *Neophocaena phocaenoides*	Chiba, Pacific Coast	**680**[c]	**1800**[c]	**810**[c]	**3300**[c]
		Seto Inland Sea	**3000**[c]	**6100**[c]	**1100**[c]	**10 000**[c]
		Nagasaki	**940**	**3700**	**510**	**5200**
		Ise Bay	**130**[c]	**790**[c]	**200**[c]	**1100**[c]
	pinnipeds					
	larga seal *Phoca largha*	Rausu, Hokkaido Coast	**21**	**23**	**5.9**	**50**
	ribbon seal *Histriophoca faciata*	Rausu, Hokkaido Coast	**23**	**25**	**27**	**75**
	northern fur seal *Callorinus ursinus*	Sanriku Coast	**93**	**130**	**93**	**320**
China	finless porpoise *Neophocaena phocaenoides*	Dongshan	**130** (99−220)	**670** (570−880)	**90** (57−130)	**890** (730−1200)
		Lusi	**37** (21−46)	**270** (220−340)	**74** (42−93)	**380** (350−430)
Philippines	long-snouted spinner dolphin *Stenella longirostris*	Sulu Sea	**2.0** (<4.0−3.1)	**32** (23−41)	**21** (19−23)	**55** (42−67)
	Fraser's dolphin *Lagenodelphis hosei*	Sulu Sea	**15** (<4.0−29)	**53** (38−68)	**26** (21−31)	**94** (89−98)
West Pacific	rough-toothed dolphin *Steno bredanensis*	Pacific Ocean	**5.7** (4.4−8.7)	**10** (6.9−16)	**6.7** (3.5−12)	**22** (17−37)
India	Indo-Pacific humpbacked dolphin *Sousa chinensis*	Bay of Bengal	**22** (11−46)	**47** (22−71)	**54** (34−100)	**120** (67−200)
	long-snouted spinner dolphin *Stenella longirostris*	Bay of Bengal	**10** (5.6−17)	**32** (21−45)	**53** (29−70)	**95** (66−130)
	bottlenose dolphin *Tursiops truncatus*	Bay of Bengal	**26** (11−29)	**44** (15−84)	**35** (16−55)	**110** (53−170)

[a] ΣBTs values were rounded. Bold: mean concentration. Ranges are given in parentheses. [b] Data reported in ref 26. [c] Data reported in ref 20.

compounds was not yet extensive.

Interestingly, compared with cetaceans collected off Japanese coastal waters, pinnipeds from Sanriku and Hokkaido coasts of Japan were found to contain lower hepatic BT concentrations. These results seem to suggest a difference in degradation capacities between cetaceans and pinnipeds as reported for PCBs (*30−32*). It was suggested that pinnipeds have a higher metabolic capacity to degrade BTs than the cetaceans (*20*). High BT residues were detected in the hair of Steller sea lion, accounting for about 25% of BT burden, which is excreted through moulting. Thus, considerable excretion of BTs in piliferous pinnipeds could also account for its lower BT levels when compared with the levels in cetaceans.

A pregnant killer whale collected off Taiji, Japan, enabled us to examine the transplacental transfer of BTs from the mother to her fetus. Concentrations of TBT and its breakdown products in liver of this pregnant killer whale and her fetus were determined, and concentration ratios in the liver of the fetus to that of its mother were calculated. The concentration ratio of ΣBTs was quite low at 0.015 (Table 3). Among TBT and its breakdown products, the concentration ratio of TBT (0.18) was found to be the highest, followed by DBT (0.009) and MBT (<0.004). In the case of methyl mercury

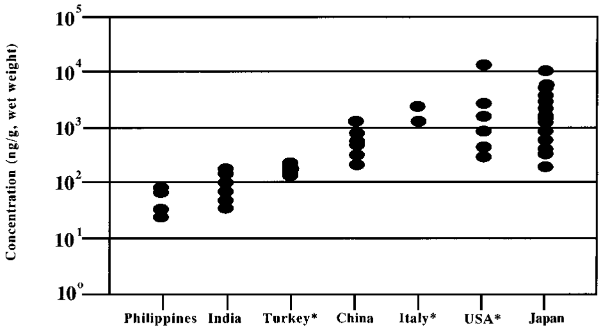

FIGURE 2. Comparison of hepatic butyltin concentrations in individual adult cetaceans from various locations. Values for Turkey, Italy, and U.S.A. were cited from refs 28, 27, and 23, respectively.

TABLE 3. Butyltin Concentrations (ng/g, wet wt) in the Liver of a Pregnant Killer Whale and Her Fetus

specimen	MBT	DBT	TBT	ΣBTs
mother (*A*)	1100	1500	150	2700
fetus (*B*)	<4.0	14	26	40
concn ratio (*B*/*A*)	<0.004	0.009	0.18	0.015

(MeHg), the estimated concentration ratio of MeHg in the liver of striped dolphin fetus to its pregnant mother was reported to be 0.17 (*33*). Transplacental transfer of PCBs and chlorinated hydrocarbon pesticides from the blubber of pregnant striped dolphin to that of her fetus showed concentration ratios of 0.38 and 0.46, respectively (*34*). The concentration ratio of ΣBTs (0.015) estimated in the pregnant killer whale examined here was found to be lower than the reported concentration ratio values of some other chemicals. These observations indicate that, in the case of BTs, the transplacental transfer from the mother to her fetus is a good deal less.

Among the BTs, DBT was predominant in most of the liver samples, followed by TBT and MBT. This pattern is similar to BT composition observed in other cetaceans previously studied, which includes harbor porpoises from the Black Sea (*28*), bottlenosed dolphins along the coasts of Italy (*27*), and stranded cetaceans along the U.S. Atlantic and Gulf coasts (*23*). These studies and present findings are indicative of similar metabolic processes among cetaceans.

Higher accumulations of TBT/BTs than DBT/BTs were detected in spinner and hump-backed dolphins collected from the Bay of Bengal, India. In contrast, the same species of spinner dolphins collected from the Sulu Sea, Philippines, revealed higher ratio of DBT/BTs than TBT/BTs. The reason for the apparent difference in BTs composition among the spinner dolphins collected from India and the Philippines remains unclear. However, higher maritime activities in the Bay of Bengal, India, than in Sulu Sea, Philippines, could be a plausible explanation.

DBT in the liver of marine mammals collected from developed countries accounted for 55−75% of total BTs detected. Whereas in samples collected from developing countries located mostly in the tropics, DBT contributed 33−57% of the total hepatic BTs. MBT/ΣBTs and DBT/ΣBTs ratios were observed to be significantly higher ($p < 0.001$, Mann-Whitney *U*-test) in mammals from developed countries, which may be indicative of greater inputs of MBT and DBT, originating from stabilizers for chlorinated polymers, etc., in coastal waters of developed nations.

Collectively, comparison of BT residue levels among marine mammals revealed higher BT concentrations in coastal than off-shore species, suggestive of greater contamination in coastal areas than in the open sea. Mammals inhabiting waters proximal to developed nations were found to have higher BT accumulation than their counterparts in the tropical waters of developing countries. This characteristic indicates a more serious pollution by BTs in marine ecosystem located around developed countries, where usage of TBT-containing products is deemed heavy. The present results also showed that BT accumulation in pinnipeds was lower than in cetaceans, confirming earlier notion that pinnipeds have greater capacity to degrade TBT in the liver and excrete BTs through molting. The present study revealed further low concentration ratio of BTs in the liver of the fetus to that of its mother killer whale indicative that BTs are less transferable from mother to fetus. Finally, significant concentrations of BTs detected in marine mammals examined necessitate further investigations that would elucidate the toxic impacts of BTs on these high trophic aquatic animals

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