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Bioaccumulation of Butyltin Compounds in Marine Mammals: The Specific Tissue Distribution and Composition

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Although organotins are notorious man-made organometallic species introduced into the aquatic environment, no investigation had been concerned with contamination of higher trophic animals such as marine mammals until the last few years. Our recent work demonstrated the detection of butyltin compounds (BTCs), including mono- (MBT), di-(DBT), and tri-butyltin (TBT) in marine mammals. This paper reviews BTC contamination in higher trophic animals, based on our recent publications. Analysis for BTCs showed significant accumulation in tissues and organs of three finless porpoises (*Neophocaena phocaenoides*) collected from Japanese coastal waters. More than 10 μg of butyltin ions per gram on a wet weight basis were detected in the liver of a porpoise collected in the semi-closed sea. Distribution of BTCs in the tissues and organs of the porpoises showed a similar pattern to several other marine mammal species: higher concentrations in liver and kidney, and lower in muscle and blubber. In addition, tissues and organs from two water birds and one sea turtle species were also analyzed for BTCs, and their concentrations and compositions were compared among the species. The results showed that the distribution of these contaminants extends widely, not only to marine mammals but also to other higher trophic species. On the other hand, the composition of the BTCs exhibited a specific profile in each species. The ratios of hepatic concentrations of DBT or MBT to TBT for marine mammals were relatively lower than those of water birds and the sea turtle, indicating that metabolism and excretion of TBT may be less efficient in the mammalian species. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

Tributyltin (TBT), used worldwide since the 1960s as antifouling agents in paints for boats and aquaculture nets, is a highly toxic chemical for some non-target species. Adverse effects in oysters and snails such as reduced reproductive capability, abnormal shell thickening and imposex have been reported in association with TBT exposure.[1, 2] Considerable attention has been focused on monitoring such lower trophic organisms. In spite of the banning or regulation of usage for TBT in some countries, contamination continues in the aquatic environment, and environmental concentrations remain high enough to warrant continued concern.[3] TBT levels in some open bays and estuaries are still close to the concentrations indicated to be toxic in some chronic toxicity tests.[4, 5]

Marine mammals such as dolphins and seals, top predators in the aquatic ecosystem, have been recorded as accumulating significant residue levels of lipophilic pollutants such as chlorinated hydrocarbons.[6, 7] Considering the wide application of TBT in the aquatic environment and the bioaccumulation potential estimated from the octanol-water partition coefficient, BTCs may be thought to occur in marine mammals. In addi-

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tion, some laboratory studies using experimental animals have demonstrated the immunotoxicity, neurotoxicity, teratogenicity and cutaneous toxicity of organotins.[8–11] Thus, despite some suggestions regarding the toxic impacts of organotins upon mammals such as rats and mice, there had been no information on contamination of marine mammals until the last few years. For the conservation and management of marine mammal populations and their ecosystems, it is essential to know whether or not they are exposed to intolerable levels of BTCs.

In our recent studies,[12, 13] an analytical procedure which removes fat from BTCs extract was developed, and enabled BTC analysis in tissues with a high fat content, such as blubber. Analysis of 12 blubber samples of eight marine mammal species collected from the open oceans and Asian coastal waters showed that the contamination by BTCs was spread on a global scale. Furthermore, BTCs in tissues and organs of three finless porpoises were quantified, and their distribution and burdens were clarified. However, the underlying mechanisms of BTC accumulation in higher trophic animals still remained unclear. Therefore, a further insight into the mechanisms of distribution and metabolism of BTCs is necessary in assessing the toxic response to exposure to the hazardous chemicals.

As a follow-up study, liver, kidney, muscle and blubber samples of a largha seal (*Phoca largha*) and a ginkgo-toothed beaked whale (*Mesoplodon ginkgodens*) collected from Japanese coastal waters in the 1990s were additionally analyzed for BTCs. Liver samples of a common cormorant (*Phalacrocorax carbo*), laysan albatross (*Diomedea immutabilis*) and loggerhead turtle (*Caretta caretta*) were also examined. In this study, the concentrations, distributions and metabolic profiles (composition ratios of TBT, DBT and MBT) of BTCs in the species were compared with those in finless porpoises. This paper presents a review of our recent work with additional results.

MATERIALS AND METHODS

All the marine mammals were collected during the period 1981–1994. Biometry of these animals, including collection year, location, body length and sex, were described in our earlier papers.[12, 13] The common cormorant and laysan albatross were obtained from Lake Biwa, Japan, and the North Pacific, respectively. The loggerhead turtle was from Tosashimizu, which is located on the Pacific coast of Shikoku Island, Japan. The animals were taken to the laboratory, dissected, and stored at $-20\,^{\circ}C$ until analysis.

The analytical method for BTCs has been described in detail elsewhere.[12] About 1–2 g of tissue was homogenized with 1 N HCl and 0.1% tropolone/acetone. The BTCs in the extracts were transferred to 0.1% tropolone/benzene, and the moisture in the solvent was removed with anhydrous Na_2SO_4. By adding propylmagnesium bromide, BTCs were propylated. For fat removal from the extract, the BTC-containing extract was added to a dry Florisil column and eluted with 20% water/acetonitrile. The eluate was then introduced to a wet Florisil column for further purification. The final extract was injected into a gas chromatograph–flame photometric detector with a tin mode filter (610 nm).

In order to check the recoveries of BTCs through the analytical procedure from tissues, 0.1 μg of butyltin chloride species dissolved in hexane was spiked into 1–2 g of the liver and blubber of a whale and subjected to the analysis. Concurrently, butyltin chloride mixture without a matrix was also prepared as a reference. Recoveries for MBT, DBT and TBT in liver matrix were $96.9 \pm 26.1\%$, $102 \pm 8.7\%$ and $91.1 \pm 13.3\%$ ($n = 3$), respectively. Besides this result, recoveries with blubber matrix were $65.3 \pm 17.3\%$ for MBT, $68.0 \pm 23.1\%$ for DBT and $55.7 \pm 5.8\%$ for TBT ($n = 4$). All the concentration data are given as nanograms of butyltin ion per gram on a wet weight basis, if not specified. The concentrations of BTCs in the samples were not corrected, considering the result of the spiking experiment.

RESULTS AND DISCUSSION

Geographical distribution

As a first step, we attempted to detect BTCs using blubber samples of marine mammals, in which persistent and lipophilic chlorinated hydrocarbons were accumulated in high concentrations.[12] Fairly reproducible recoveries of BTCs in the blubber matrix made the data reliable, and led to the first confirmation of BTC contamination in the bodies of animals. Also, comparison of BTCs concentrations in the blubber of marine mammals, collected from various

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locations, enabled us to depict roughly the geographical distribution of the contamination. The result was that BTC concentrations were apparently higher in the species collected from Japanese coastal waters, and showed lower levels in those from open seas. The highest residue level was found in a finless porpoise from an inland sea. This heavy contamination of BTCs may be due to sources from busy shipping and aquacultural activities, and to slow exchange of seawater in this semi-closed sea. Detection of BTCs in the open-ocean species suggested worldwide pollution, despite their lower residue levels.

Tissue distribution and residue level

As a next step, tissue distribution of BTCs were examined using finless porpoises[13] which had shown relatively higher concentrations in the previous blubber analysis.[12] BTCs were also detected in all the other tissues and organs of porpoises as well as blubber (Table 1). The highest BTC concentration was found in the liver at a parts-per-million (ppm) level on a wet weight basis, followed by the kidney. Specifically, a finless porpoise collected in the Setonaikai (Seto Inland Sea) was highly contaminated, with 10.2 and 3.2 $\mu g\ g^{-1}$ BTCs in the liver and kidney, respectively. Residue levels of BTCs in the blubber and muscle were lower than the hepatic and renal concentrations. This distribution pattern appeared to be similar to those in the largha seal and ginkgo-toothed beaked whale, although the residue levels in each tissue of the latter species were much lower (Table 1). Furthermore, the contents of the intestine in a finless porpoise also contained significant levels of BTCs, suggesting process of bioaccumulation through diet.

It has been reported that BTCs in laboratory rodents are less bioaccumulated due to rapid metabolism. TBT metabolism is considered in association with the mixed-function oxygenase (MFO) system. Hence, the capacity to accumulate BTCs in rodents has been supposed to be much lower than in fish, which have lower MFO activities.[14] The present results suggest that the assumption may not be true for marine mammals. The high accumulation of BTCs in finless porpoises might partly be due to the lower MFO activities in marine mammals than those in rodents, in addition to the proximity of their habitat to BTC sources. It has been shown that in cetaceans activities of cytochrome *P*-450, which is inducible by phenobarbital (PB), are lower than those in rats.[15] Furthermore, PB-inducible forms of cytochrome *P*-450 in rats were found to catalyze the hydroxylation and dealkylation of BTCs.[16,17] On the other hand, it has also been reported that high exposure to TBT inhibits MFO activities in fish liver.[18] The MFO inhibition in the cetacean liver may accelerate TBT accumulation. A laboratory experiment, using rodents to examine the toxicities of BTCs, showed that external malformations were caused at a few micrograms of DBT residues per gram of liver.[19] Although caution is needed to extrapolate the experimental results in rodents to those in cetaceans, this may suggest that the finless porpoises were exposed to BTCs at toxicologically significant levels. Furthermore, TBT has also been reported to be an endocrine disrupter which shares common metabolic pathways with hormones,[20] and an immunosuppressor which gives rise to thymus atrophy.[21]

The distribution pattern of BTC concentrations in marine mammals appeared to be different from those of chlorinated hydrocarbons such as PCBs and DDTs, which were retained preferentially in blubber (Fig. 1). In contrast, the pattern of BTCs was rather similar to that of organic (methyl)mercury in marine mammals, showing larger residues in liver.[23] This suggests that accumulation of BTCs is less dependent on their affinity to lipids in tissues and organs. A recent review of the transport mechanism of methylmercury in experimental animals indicated that it forms a complex with glutathione and the complex plays a key role in the tissue distribution.[24] The fact that the distribution of BTCs is similar to that of methylmercury may suggest a higher affinity to sulfhydryl groups such as glutathione rather than to lipids.[13] Our group has recently found significant contamination in the hair of seals[25] and the feathers of birds,[26] which contain large amounts of cysteine with sulfhydryl groups.

BTC compositions

BTC compositions (relative concentrations) in the porpoise samples are different from one tissue/organ to another (Fig. 2). DBT comprised a higher proportion in the liver (55–71%) and blood (78%) in comparison with other tissues and organs. In contrast, DBT concentrations

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Table 1. Concentrations (ng of butyltin ion per g, wet weight basis) of BTCs in tissues and organs of higher trophic animals

Sample no.	Sampling year	Sampling location	Tissues and organs	MBT	DBT	TBT	BTCs
Finless porpoise							
1[a]	1981	Chiba	Muscle	18	12	99	129
			Blubber	91	<5	36	127
			Liver	130	790	200	1120
			Kidney	47	150	130	327
			Heart	19	15	140	174
			Lung	12	14	41	67
			Stomach	11	13	34	58
2[a]	1985	Seto Inland Sea	Muscle	67	68	260	395
			Blubber	210	74	460	744
			Liver	3000	6100	1100	10200
			Kidney	340	870	2000	3210
			Heart	130	140	330	600
			Lung	44	110	1300	1450
			Stomach	150	250	600	1000
			Brain	350	460	2200	3010
			Testis	120	390	770	1280
3[a]	1994	Ise Bay	Muscle	22	38	420	480
			Blubber	73	20	120	213
			Liver	680	1800	810	3290
			Kidney	55	340	510	905
			Heart	12	41	640	693
			Lung	90	59	160	309
			Stomach	18	64	130	212
			Brain	9	40	350	399
			Adrenal gland	130	350	250	730
			Spleen	33	98	69	200
			Bone[c]	14	21	33	68
			Esophagus	19	36	49	104
			Pancreas	72	130	220	422
			Intestine	9	70	66	145
			Urinary bladder	26	67	56	149
			Eye	<5	12	35	47
			Blood	82	640	95	817
			Intestinal contents	6	47	150	203
Ginkgo-toothed beaked whale							
4[b]	1993	Japan Sea	Muscle	20	8	19	47
			Blubber	20	10	33	63
			Liver	120	130	76	326
			Kidney	66	34	42	142
Largha seal							
5[b]	1992	Western coast of Hokkaido	Muscle	14	6	20	40
			Blubber	14	1	4	19
			Liver	96	200	32	328
			Kidney	27	55	28	110
Common cormorant							
6	1993	Lake Biwa	Liver	150	180	19	349
Laysan albatross							
7	1989	North Pacific	Liver	80	62	8.5	151
Loggerhead turtle							
8	1990	Tosashimizu	Liver	68	60	2.4	130

[a] Data are from Ref. 13.
[b] Data on blubber of the animals are from Ref. 12.
[c] The bone marrow of a rib was analyzed.

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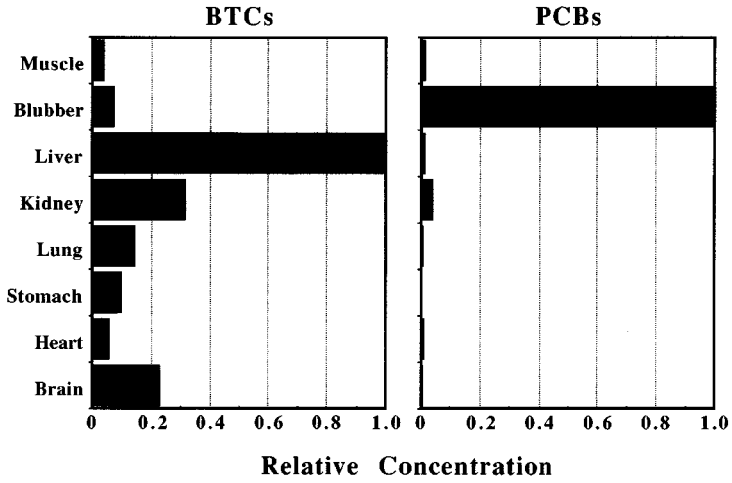

Figure 1 Comparison of relative concentrations between BTCs and PCBs in tissues and organs. The highest concentration is regarded as 1.0. Data of BTCs are from sample no. 2 (see Table 1). Data of PCBs are from Ref. 22.

were relatively low in the blubber. These differences may be caused by the tissue- and organ-specific metabolic capacity subsequent to TBT exposure and/or the specific kinetics of each butyltin species, depending on their physicochemical parameters and bioavailabilities. The results also exhibited a similar pattern of BTC composition among the other marine mammals examined (Table 1). It is noteworthy that, in the intestinal content of the animal collected from Ise Bay in 1994, TBT was the dominant species among the BTCs, suggesting a higher uptake of TBT through diet. If release of TBT to the environment had decreased or stopped, the proportion of degradation products (DBT and MBT) of TBT would be higher. Several studies have given an indication of degradation of TBT to DBT and further to MBT in the aquatic environment.[27] The larger proportion of TBT in the intestine content indicates continuous inputs of TBT in Ise Bay. In Japan, TBT is still being used as an antifouling agent for particular vessels, although the production and import of 13 TBT species were regulated in 1990.[28]

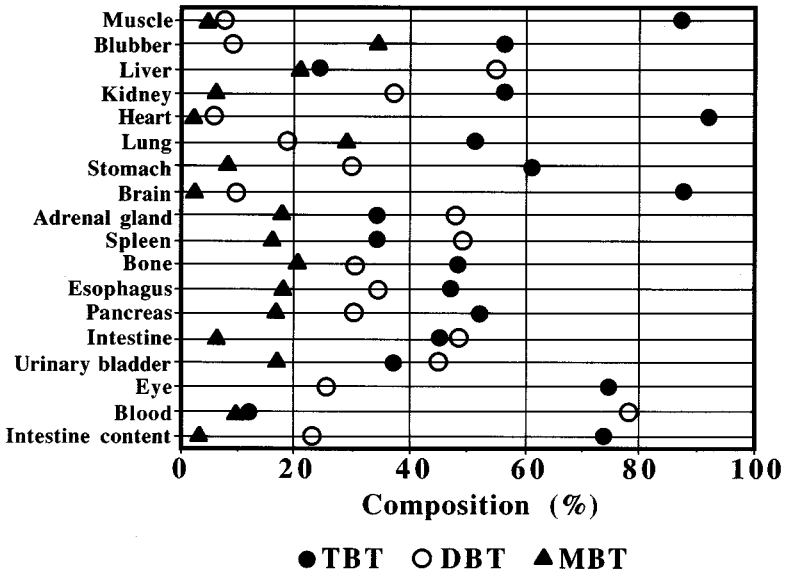

Figure 2 Butyltin compositions in tissues and organs of a finless porpoise collected from Ise Bay.

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Burden and biomagnification factor

BTC burdens contained in a finless porpoise from Ise Bay were calculated from the BTC concentrations in each tissue/organ and their individual weights. Consequently, 16.5 mg of BTCs (MBT, 2.14 mg; DBT, 3.36 mg; TBT, 11 mg) were estimated to be accumulated in the whole body. The residue of TBT at the larger proportion, as well as the BTC composition in the contents of the intestine, implies again that release of TBT from some sources is in progress. Moreover, this may also mean a lower metabolic potential for TBT in this animal, as suggested from the higher TBT concentrations associated with the low MFO activities.

Regarding the reservoirs of total BTCs, muscle (54.2%), blubber (15.5%) and liver (18.7%) played an important role. While the largest portion of TBT (71.2%) was found in muscle, MBT and DBT were retained predominantly in blubber (41.0%) and liver (50.2%), respectively (Fig. 3). Differences in the reservoirs among BTCs also indicate the presence of specific metabolic activities or kinetics of accumulation in each tissue and organ, as was suggested by the proportions of the concentrations (i.e. the composition).

Using the total burden (11.0 mg) of TBT and the body weight (40.3 kg), an average concentration in the whole body was calculated to be 270 ng g^{-1} on a wet weight basis. Therefore, a biomagnification factor (BMF) could be estimated from the concentration of the whole body divided by TBT (150 ng g^{-1} on a wet weight basis) in the intestine content as feed, assuming that the moisture content for the whole body is similar to that of the contents of the intestine. The BMF value (1.8) of porpoise appeared to be almost five times higher than that (0.26–0.38) of the red sea bream (*Pagrus major*), which had been previously obtained from a laboratory experiment.[29] The comparison of BMFs implies that the porpoise accumulates TBT more efficiently than the fish species, although differences in the experimental conditions should be taken into account.

Interspecies comparison

In order to clarify the accumulation and metabolic capabilities of BTCs in marine mammals, the hepatic concentrations and compositions were compared with those in water birds, sea turtles and other marine species. The comparison showed that residue levels in finless porpoises, which were primarily coastal inhabitants, were very high, and those in the other higher trophic animals were one order of magnitude lower (Fig. 4).

Regarding the metabolic capabilities of BTCs, the composition patterns in the water birds and sea turtle seemed to be different from those in marine mammals. The common cormorant, lay-

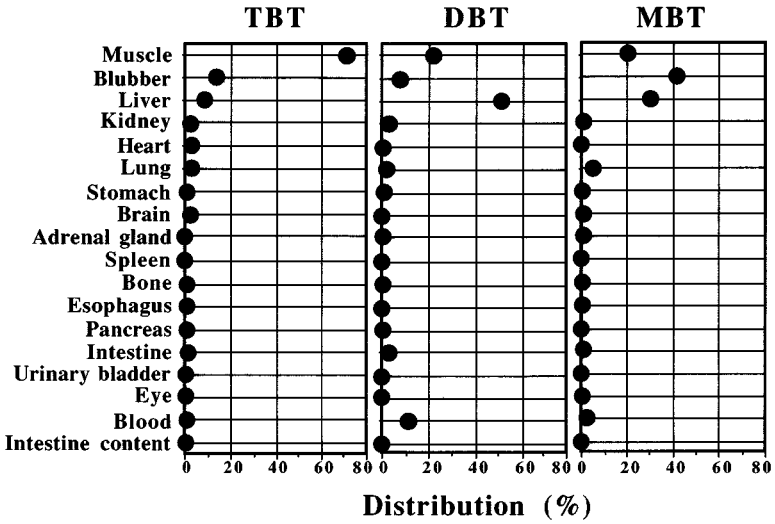

Figure 3 Distribution of butyltin burdens in the tissues and organs of a finless porpoise collected from Ise Bay. The distribution (%) was estimated by dividing the burdens of the butyltin species in each tissue and organ by the total burden in the whole body.

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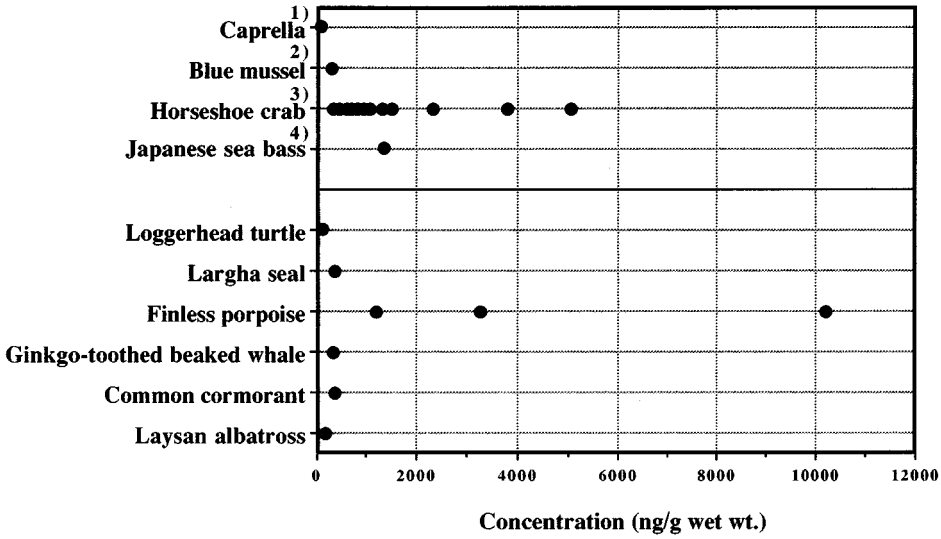

Figure 4 Interspecies comparison of butyltin concentrations in livers. 1) Caprella: whole body (Iwata *et al*., unpublished data). 2) Blue mussel: soft tissue (Ref. 30). 3) Data from Ref. 31. 4) Data from Ref. 32.

san albatross and loggerhead turtle showed larger amounts of butyltin metabolites (DBT and MBT) compared with TBT than marine mammals. Particularly, the loggerhead turtle was found to include TBT at a lower concentration than DBT and MBT. On the contrary, lower trophic animals (caprella, blue mussel, horseshoe crab and sea bass) are likely to exhibit low proportions of the butyltin metabolites to TBT, which are comparable with marine mammals. As an example, the ratios of DBT to TBT are presented in Fig. 5. These results may suggest that the metabolic capabilities of marine mammals are lower than those of the birds/turtle, and equal to or higher than those of the lower trophic animals. It should also be noted that marine mammals, including the seal, whale and porpoise, exhibited rather similar ratios, although the BTC concentrations, collection years and locations were different. These results may indicate that the differences in BTC compositions between marine mammals and the other higher trophic animals more

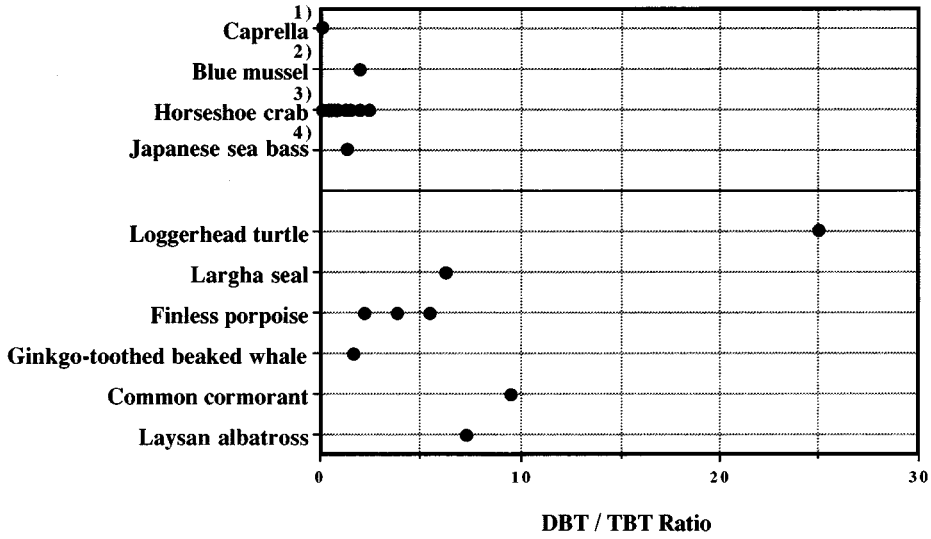

Figure 5 Interspecies comparison of DBT/TBT ratios in livers. 1) Caprella: whole body (Iwata *et al*., unpublished data). 2) Blue mussel: soft tissue (Ref. 30). 3) Data from Ref. 31. 4) Data from Ref. 32.

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strongly reflect their metabolic capabilities rather than the amounts of BTCs to which the animals were exposed.

Conclusions

This study clearly shows significant accumulation of BTCs in all of the tissues/organs of marine mammals. The elevated BTC concentrations in the top predators of the aquatic ecosystem indicates the transfer of these compounds through the food chain and may have some impact on marine mammals. The higher concentrations of BTCs, the larger burden of TBT than the dealkylated metabolites, and the interspecies comparison of DBT (or MBT)/TBT ratios in liver may suggest that marine mammals have lower metabolic capabilities than other higher trophic animals. Further studies are necessary to determine the bioaccumulation processes of BTCs and related factors in marine mammals which would allow us to assess their ecotoxicological risk on the basis of a better understanding.

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