estimated for DDTs, HCHs, CHLs, and PCBs are much lower than acceptable daily intake (ADI) values proposed by FAO/WHO (1996).

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Contamination of Organotin Compounds in Finless Porpoises (*Neophocaena phocaenoides*) Stranded along Coastal Waters of Japan and Hong Kong

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Abstract. Organotins (OTs) including butyltin (BTs), octyltin (OcTs) and phenyltin (PTs) compounds were determined in various tissues and organs of finless porpoises stranded along coastal waters of Japan and Hong Kong. OTs were detected in all the samples analyzed. BTs were detected at the highest concentration in the liver and the lowest in fat tissues. On the other hand, triphenyltin (TPT) concentrations were rather uniform among the tissues and organs, suggesting the difference in accumulation mechanism between BTs and PTs. In the brain, trisubstituted OTs, tributyltin (TBT) and TPT, were accumulated as dominant compounds. OTs concentrations increased with growth, and no sex difference in the accumulation of OTs was observed. An estimated transplacental transfer rate and OTs concentration in milk indicate the low gestational and lactational transfer of these compounds. Finless porpoise in Hong Kong waters accumulated the highest hepatic concentrations of OTs , followed by those in Seto Inland Sea, Ise and Mikawa Bays and Pacific coast of Japan. Concentrations of BTs in the livers of finless porpoises from Ise and Mikawa Bays analyzed in this study were lower than those reported on same species stranded along Ise Bay in 1994, reflecting the reduction of environmental release of TBT. However, from 1998 to 2003, no significant decreasing trend in OTs concentrations was observed in the liver samples of finless porpoises from Ise and Mikawa Bays. Concentrations of BTs found in some livers of finless porpoises exceeded the toxic thresholds of hepatotoxicity and immunotoxicity reported in *in vivo* and *in vitro* studies. These results suggest the continuous contamination by OTs and its toxic risk on finless porpoises in these areas.

Introduction

OTs, particularly TBT and TPT, have been used in paints for ships and aquaculture nets. However, these compounds are toxic to non-target aquatic life even at low concentration and it has been well documented that TBT cause imposex in gastropods (Horiguchi et al. 1995). Embryotoxic effects on various aquatic organisms and immunotoxic effects on mammals have also been caused by these compounds (Fent 1996; Ema & Harazono 2001; Thomas et al. 2004). Cetaceans are the top predators of marine ecosystem and accumulate various contaminants to high levels through food chain. Finless porpoise, *Neophocaena phocaenoides*, has their habitat in shallow (less than 50m deep) and coastal waters (Folkens et al. 2002). Therefore, they are affected by human and industrial activities such as bycatch and water pollution in coastal environments. Our previous studies showed that finless porpoises in Japan and Hong Kong accumulated BTs at very high concentrations (Iwata et al. 1995; Takahashi et al. 2000). However, accumulation of PTs and OcTs and temporal trend of OTs contamination in finless porpoise have not been documented well.

In this study, concentrations of BTs, PTs and OcTs were determined in various tissues and organs of finless porpoises stranded along coastal waters of Japan and Hong Kong to elucidate their body distribution and accumulation properties, understand their contamination status and temporal trend and evaluate their toxic risks on hepatotoxicity and immunotoxicity.

Materials and Methods

Samples. Liver samples were collected from finless porpoises stranded along coastal waters of Japan including Ise and Mikawa Bays (*n*=24, 1994-2004), Seto Inland Sea (*n*=12, 1999-2000) and Pacific coast (*n*=5, 1999-2000) and Hong Kong (*n*=4, 2000-2001). Various tissues and organs including liver, kidney, brain, spleen, pancreas, testis, epididymis, muscle, blubber and melon were collected from finless porpoise stranded at Ise Bay in 1994. All samples were stored in polyethylene bags at –20°C in the laboratory until chemical analysis.

Chemical analysis. Eight species of OTs, mono- (MBT), di- (DBT) and tributyltins (TBT), di- (DPT) and triphenyltins (TPT) and mono- (MOcT), di- (DOcT) and trioctyltins (TOcT), were measured in this study. These OTs in the tissues/organs were extracted by 1N HBr methanol/ethyl acetate and other organic solvents, and then ethylated by 5% tetraethyl sodium borate. Fat in the extract was saponified by 1M KOH, and the final solution was cleaned up by SEP-PAK Florisil colomn. OTs were identified and quantified by GC-MS (EI-SIM) using deuterium-labeled internal standard (d_9-MBT, d_{18}-DBT, d_{27}-TBT, d_5-MPT, d_{10}-DPT, d_{15}-TPT, d_{17}-MOcT, d_{34}-DOcT, d_{51}-TOcT and d_{36}-tetrabutyltin).

Results and Discussion

Body distribution. Concentrations of BTs showed considerable variation among tissues and organs (Table 1). The highest concentration was observed in the liver. The proportions of DBT and MBT were found to be higher than TBT in the liver and kidney, suggesting metabolism of TBT in these organs. On the other hand, PTs concentrations were rather uniform and the proportions of TPT to total PTs were more than 90% in all the organs analyzed. These results suggest the different accumulation and metabolic behavior of BTs and PTs. TBT and TPT were detected in relatively high concentrations in the brain, implying that these OTs can pass through the blood-brain barrier. This suggests that these compounds may have high affinity to polar lipids in the brain. Significant accumulation of TBT and TPT may cause neurotoxic effects in finless porpoises.

This study is the first report on the detection of OcTs in marine mammal specimens. MOcTs and DOcTs have been used for industrial applications such as PVC stabilizers and polymer catalysts. Detection of OcTs in finless porpoise indicates that OTs used in terrestrial environments are accumulated in aquatic life, although OcTs concentrations were much lower than BTs in finless porpoises. OcTs showed organ specific accumulation. Relatively high concentrations were found in the epididymis and kidney, and hepatic concentration was below the detection limit. Further studies are needed to clarify the accumulation pattern of OcTs in marine mammals.

Table 1. Concentrations (ng/g wet wt.) of OTs in finless porpoise from Ise Bay.

Sample No.	Sampling Date	BL(cm)	Sex	Tissue	MBT	DBT	TBT	DPT	TPT	MOcT	DOcT	TOcT	ΣBTs	ΣPTs	ΣOcTs	ΣOTs
940823-2	1994.08.23	152	♂	liver	260	2200	460	7.0	62	<0.3	<0.2	<0.5	2900	69	-	3000
				kidney	64	540	190	1.7	52	2.0	6.9	<0.5	790	54	8.9	850
				pancreas	65	53	34	1.5	15	0.5	3.2	<0.5	150	17	3.8	170
				spleen	32	59	69	1.7	26	<0.3	2.7	<0.5	160	27	2.7	190
				muscle	6.7	9.4	84	0.6	23	3.2	3.1	<0.5	100	24	6.2	130
				testis	12	20	50	0.6	20	2.1	1.6	<0.5	83	20	3.6	110
				epididymis	26	30	67	0.9	23	30	15	<0.5	120	24	45	190
				blubber	2.5	6.0	38	NA	24	<0.3	NA	<0.5	47	24	-	70
				melon	2.2	10	33	NA	31	9.3	NA	<0.5	46	31	9.3	86
				brain	11	46	130	0.8	56	<0.3	0.7	<0.5	190	57	0.7	250

Age trend, sex difference and maternal transfer. Concentrations of OTs increased with growth throughout their life (Figure 1). Previous studies on cetaceans reported that BTs concentrations in the liver increased until maturity and then reached steady state (Kim et al. 1996a; Kannan et al. 1997). Difference in OTs levels between finless porpoise and other cetaceans may be due to species-specific metabolic capacity and/or biological/ecological factors such as feeding habitat. On the other hand, lower concentrations of OTs in young porpoises may reflect decreasing exposure to OTs after the restriction on usage of OTs since the late 1980s.

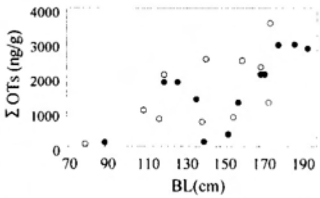


Fig. 1 Relationship between body length and hepatic concentrations of Σ BTs, Σ PTs and Σ OTs. (●: male ○: female)

No sex difference was found in the accumulation of OTs (Figure 1). OTs concentration in milk (20ng/g wet wt.: data not shown) was relatively low compared to that reported for PCBs (507ng/g wet wt.; Kawai et al. 1988), while the estimated transplacental transfer rate of OTs (10.0±7.9%) was comparable with that of PCBs (4.0%; Tanabe et al. 1982). Hence, low maternal transfer of OTs would result from their low lactational transfer.

Contamination status and temporal trends. Finless porpoise population of Hong Kong accumulated the highest concentrations of OTs in the liver, followed by those in Seto Inland Sea, Ise and Mikawa Bays and Pacific coast of Japan (Table 2). This result suggests significant contamination by OTs in Hong Kong where intensive industrial and shipping activities are of concern. Concentration levels of BTs in the livers of finless porpoises from Japanese coastal waters found in this study were lower than those reported in the 1980s and early 1990s (Table 2), reflecting the regulation on TBT usage since the late 1980s. However, from 1998 to 2003, no significant decrease in OTs concentrations was observed in finless porpoises from Ise and Mikawa Bays (data not shown), suggesting slower or no substantial decline of contamination in recent years. In Hong Kong, no significant decline was observed in the hepatic levels of BTs in this species from 1997 to 2001 despite the regulation of TBT usage in 1992 (Table 2). Hong Kong is an international port with high transportation of vessels (Marine Department of Hong Kong SAR 2005), suggesting continuous release of OTs from international shipping activities to marine environment in this area.

Table 2. OTs concentration (ng/g wet wt) in the livers of finless porpoises from Japan and Hong Kong.

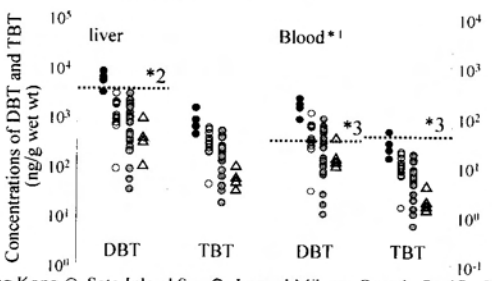
Location	Year	n ♂	n ♀	BL (cm)	MBT	DBT	TBT	DPT	TPT	ΣBTs	ΣPTs	ΣOTs
Seto Inland Sea	1985*1	1		162	3000	6100	1100	NA	NA	10200	-	-
	1999-2001	6	2	156 (142-171)	320 (25-1300)	1500 (83-3100)	270 (41-560)	6.0 (<0.1-6.7)	31 (10-72)	2100 (150-4900)	33 (11-72)	2100 (160-5000)
Ise and Mikawa Bays	1994*1		1	139	680	1800	810	NA	NA	3300	-	-
	1998-2003	8	7	166 (141-195)	420 (43-1800)	1200 (35-2900)	200 (38-490)	4.0 (0.2-9.4)	25 (8.0-62)	1900 (130-3500)	29 (8.2-71)	1900 (140-3600)
Pacific coast	1981*1	1		151	130	790	200	NA	NA	1120	-	-
	1999-2000	3	1	168 (143-181)	55 (21-91)	480 (280-900)	59 (38-100)	NA	15 (7.8-25)	600 (340-1100	15 (7.8-25)	610 (350-1100)
Hong Kong	1996-1997*2	1	3	151 (137-168)	980 (200-1100)	2500 (670-4700)	600 (200-1300)	NA	NA	4100 (1100-8100)	-	-
	2000-2001	3	1	154 (145-163)	930 (240-900)	5200 (2200-5700	800 (370-1300)	190 (44-270)	340 (200-400)	6900 (3000-7900)	530 (240-670)	7500 (4100-11000)

Concentration (ng/g wet wt.)

*1: Iwata et al., 1995 *2: Takahashi et al., 2000

Comparison of BTs concentrations with threshold of various toxicities. Hepatic concentrations of DBT detected in some finless porpoises from Hong Kong exceeded the toxic thresholds of hepatotoxicity (Ueno et al. 1994) (Figure 2), indicating high toxic risk by BTs to finless porpoises. In addition, blood concentrations, which were estimated from the ratio of blood to hepatic concentration (1:25) (Takahashi & Tanabe 2006), in some finless porpoises exceeded the threshold for reduction of lymphocyte proliferation in Dall's porpoises (Nakata et al. 2002) (Figure 2). Continuous monitoring of OTs accumulation in finless porpoises together with epidemiologic and pathologic studies are needed for their conservation.

Figure 2. Comparison between concentrations of TBT and DBT in the liver and



● : Hong Kong ○: Seto Inland Sea ◎: Ise and Mikawa Bay △: Pacific Coast

blood of finless porpoises from different areas and threshold of various toxicities.

*1: Blood concentrations were estimated from hepatic concentrations by the ratio of blood to hepatic concentration (1:25) (Takahashi and Tanabe. 2006)

*2: In vivo hepatotoxicity in mice. >2600ng/g (Ueno et al. 1994)

*3: In vitro test for reduction of lymphocyte proliferation in Dall's porpoises. [TBT] 44ng/g [DBT] 33ng/g (Nakata et al. 2002)

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Contamination Status and Accumulation Profiles of Organotins in Southern Sea Otters (*Enhydra lutris nereis*) Found Dead along California Coast

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Abstract Organotin compounds (OTs) including butyltins (MBT, DBT, TBT), phenyltins (DPT, TPT) and octyltins (MOcT, DOcT, TOcT) were determined in the liver of adult female southern sea otters (*Enhydra lutris nereis*) found dead along the California coast during 1992-2002. In addition, sea otters found dead along the coasts of Washington State, Alaska and Russia were also analyzed for comparison. OTs were detected in all the liver samples analyzed. Total concentrations of OTs in sea otters of California ranged from 43-8200 ng/g on a wet wt basis. The order of the percentage of OTs were total butyltins ≫ total octyltins ≧ total phenyltins. Very high concentrations of BTs were found in some specimens with infectious diseases, although statistically significant difference in the hepatic concentrations of BTs was not observed between the non-infected and infected specimens. Total BTs concentrations in sea otters significantly decreased since the 1990s. The half-life of TBT estimated from the hepatic concentrations of sea otter was 3.01 years, suggesting slow degradation or continuous exposure to TBT after the ban. BTs concentrations in sea otters from California were significantly higher than those found along Kamchatka peninsula and in pinnipeds collected along the California coast. Percentage of BTs (average 79%) in ΣSn concentrations in sea otters were similar to those of cetaceans, but higher than those of pinipeds, which may be due to lower metabolic capacity to degrade OTs in sea otters than pinipeds. Concentrations of TBT and DBT found in few infected specimens were close to or even exceeded the threshold levels of toxic effects reported from *in vivo* and *in vitro* tests, suggesting high risks for sea otters.

Introduction

Organotin compounds (OTs) are used all over the world for various purposes such as antifouling agent, PVC stabilizer, catalyst etc. Recent studies showed that OTs are bioaccumulated even in high trophic level organisms such as marine mammals. Anticipating adverse biological effects and contamination of OTs, use of tributyltin (TBT) as a component in paints for ships is restricted in U.S.A. since 1988.

Southern sea otters (*Enhydra lutris nereis*) live along California coastal waters. Their populations slightly decreased due to commercial hunting in the 18th and 19th centuries. Under the protection of the international Fur Seal Treaty of 1911, population began to recover. Nevertheless, they have made a slower than expected recovery (Estes 1990). They were listed as threatened by the U.S. Fish and Wildlife service in 1977. After a decade of population growth from mid 1980 to the mid 1990s, the population decreased again slowly in the late 1990s, which was attributed to high adult mortality rates, with infectious diseases as the major cause of death (Thomas & Cole 1996). High prevalence of diseases has been hypothesized as due to their weakened immune systems resulting from exposure to toxic contaminants. OTs, especially TBT and dibutyltin (DBT), are well known as immunotoxic chemicals (Nakata et al. 2000). Previous