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Contamination status and accumulation profiles of organotins in sea otters (*Enhydra lutris*) found dead along the coasts of California, Washington, Alaska (USA), and Kamchatka (Russia)

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Abstract

Organotin compounds (OTs) including mono- to tri-butyltins, -phenyltins, and -octyltins were determined in the liver of adult sea otters (*Enhydra lutris*) found dead along the coasts of California, Washington, and Alaska in the USA and Kamchatka, Russia. Total concentrations of OTs in sea otters from California ranged from 34 to 4100 ng/g on a wet weight basis. The order of concentrations of OTs in sea otters was total butyltins $\gg$ total octyltins $\geqq$ total phenyltins. Elevated concentrations of butyltins (BTs) were found in some otters classified under 'infectious-disease' mortality category. Concentrations of BTs in few of these otters were close to or above the threshold levels for adverse health effects. Total butyltin concentrations decreased significantly in the livers of California sea otters since the 1990s. Based on the concentrations of organotins in sea otters collected from 1992 to 2002, the half-lives of tributyltin and total butyltins in sea otters were estimated to be approximately three years.

Keywords: Organotin compounds; Sea otters; Infectious diseases; California coast; Half-lives

1. Introduction

Organotin compounds (OTs) are organometallic chemicals characterized by the presence of one or more carbon–tin bonds (C–Sn). Organotins have been used in a wide variety of applications all over the world. Especially, tributyltin (TBT) and triphenyltin (TPT) were commonly used as antifouling agents to prevent the attachment of barnacles and slime on boat hulls and fishing nets. Contamination of dibutyltin (DBT), monobutyltin (MBT), diphenyltin (DPT), dioctyltin (DOcT), and monooctyltin (MOcT) arise from their use in PVC pipes as stabilizers and synthetic catalysts in silicone resins and polyurethane forms. Thus, OT compounds have been released into aquatic and terrestrial environments. It has also been documented that OTs are bioaccumulative chemicals. Studies have shown that OTs bioaccumulate even in higher-trophic level aquatic organisms such as marine mammals (Tanabe, 1999). Imposex in gastropods is a well known toxic effect caused by OTs (particularly TBT), in addition to immunotoxic, hepatotoxic, and neurotoxic effects in fish and mammals. Adverse biological effects and contamination by OTs resulted in the restriction of use of TBT in anti-fouling paints in the USA since 1988.

Mustelids such as otters and mink are aquatic mammals and are sensitive to chemical contamination (Kannan et al., 1998). Sea otters (*Enhydra lutris*) are the smallest of marine mammals with lengths varying from 100 to 130 cm. Sea otters lack blubber and the milk is low fat content

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(Kajiwara et al., 2001). Sea otters consume foods at a high rate of between 23 and 33% of their body weight every day (VanBlaricom and Estes, 1988). Sea otters eat a variety of sessile and slow-moving benthic invertebrates, which are known to accumulate high concentrations of BTs (Kannan et al., 1998). Sea otters, particularly adult female sea otters exhibit localized movement patterns (Ralls et al., 1996). Because of these characteristics, sea otters are considered as good bioindicators for monitoring local contamination by anthropogenic pollutants.

Historically, sea otters were distributed throughout the northern Pacific coastline. However, due to commercial hunting in the 18th and 19th centuries, sea otters were extirpated from most of their historical range (Kenyson, 1969). Under the protection of the International Fur Seal Treaty of 1911, sea otter populations began to recover and re-colonize their historical range. Southern sea otters (*Enhydra lutris nereis*) live along California coastal waters. Southern sea otters have made a slower than the expected recovery (Estes, 1990). In 1977, southern sea otters were listed as threatened by the US Fish and Wildlife Service. After a decade of population growth from the mid 1980s to the mid 1990s, the population of southern sea otters exhibited a slow decline in the late 1990 s. The decline was attributed to high adult mortality rates, with infectious disease as the major cause of death (Estes et al., 2003). Detailed necropsies of southern sea otters collected during 1992–1995 revealed that a high percentage of mortality was due to infectious diseases (Thomas and cole, 1996). Postmortem examination by Kreuder et al. (2003) showed that protozoal encephalitis, acanthocephalan peritonitis, shark attack, and cardiac disease were identified as common causes of death. High prevalence of diseases in southern sea otters has been hypothesized to be due to their weakened immune system resulting from exposure to toxic contaminants (Kannan et al., 1998, 2004, 2006a, 2006b, 2007; Nakata et al., 1998). OTs, especially TBT and DBT, are well known as immunotoxic chemicals that are distributed widely in aquatic ecosystems (Nakata et al., 2002). Previous studies (Kannan et al., 1998; VanBlaricom and Estes, 1988) showed that sea otters accumulate butyltins (BTs) at high concentrations and the biomagnification of BTs in sea otters results from their high diet intake rates. In addition, earlier studies showed significantly greater BT concentrations in sea otters that died of infectious diseases than those that died of non-infectious causes. However, earlier studies analyzed only BTs in southern sea otters that died during 1992–1996 (Kannan et al., 1998). Studies examining contamination by phenyltins (PTs), and octyltins (OcTs) and also temporal changes in butyltin contamination after 1996 in southern sea otters are not available. Furthermore, spatial difference in OT concentration in sea otters collected from Washington and Alaska in the USA and Kamchatka (Russia) is not known.

In this study, OTs were analyzed in liver tissue from southern sea otters found dead along the central California coast to elucidate; (1) contamination status and temporal trend of OTs, including BTs, PTs, OcTs, (2) spatial comparison of OTs among the coasts of California, Washington, Alaska and Kamchatka, (3) accumulation profiles of OTs in comparison with other marine mammal species such as pinnipeds from the California coast, (4) hazard assessment for DBT and TBT in view of immunotoxicity and hepatotoxicity in sea otter. The relationship between infectious diseases in sea otters and contamination by OTs was also examined.

2. Material and methods

2.1. Sample collection

Southern sea otters found dead in three locations along the California coast from 1992 to 2002 were collected. Sampling locations in California are shown in Fig. 1. A previous study (Kannan et al., 1998) showed that adult female sea otters accumulate high concentrations of BTs. Therefore, all sea otters analyzed from California were adult females in this study. Postmortem examinations were performed at the US Geological Survey's National Wildlife Health Center (NWHC) in Madison, Wisconsin, for the determination of cause of death (COD). A variety of tissues from sea otters were fixed in 10% buffered formalin for histopathology, and paraffin embedded, stained by hematoxylin and eosin, and examined by light microscopy. Selection of other diagnostic clinical laboratory tests was based on the history and gross lesions and included microbiologic, virologic and parasitologic procedures. The COD was classified, based on necropsy findings, into one of four categories: emaciation, infectious disease, other, and trauma. Each class is further divided into more specific subclasses. Further details of COD are given elsewhere (Kannan et al., 1998; Thomas and Cole, 1996). Twenty eight samples of adult female sea otters were selected for analysis of OTs in this study. We grouped sea otters that died from infectious disease into an 'infected' group ($n = 15$), and the otters that died from all other causes into an 'Uninfected' group ($n = 13$).

In addition to sea otters from California, to examine the spatial difference in OTs contamination, sea otters found dead along coasts of Washington (*Enhydra lutris kenyoni*) (female; $n = 3$) and Alaska (*Enhydra lutris kenyoni*) (male; $n = 1$, female; $n = 3$) in the USA, and from Kamchatka Peninsula (*Enhydra lutris lutris*) (female; $n = 5$) in Russia examined at NWHC were also analyzed. Sampling locations are shown in Fig. 1.

2.2. Chemical analysis

Analyses of organotin compounds (OTs) including mono to tri-butyltins (MBT, DBT, and TBT), -phenyltins (DPT and TPT) and -octyltins (MOcT, DOcT, and TOcT) were conducted following the method described by Iwamura et al. (2000) with slight modification.

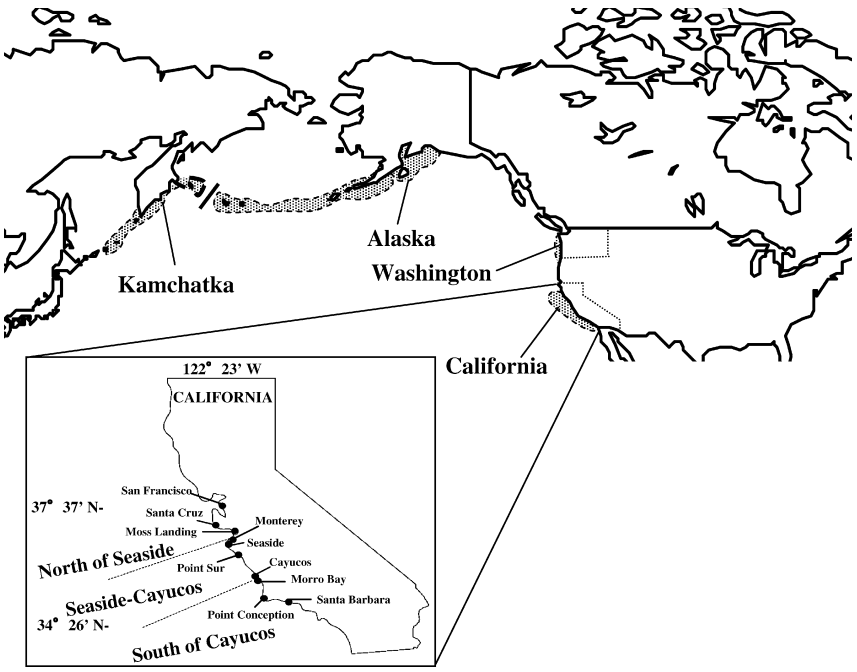

Fig. 1. Map showing sampling locations of sea otters from California, Washington, Alaska (USA), and Kamchatka (Russia).

Approximately 2 g of liver was placed in vials and spiked with 50 ng of internal standards including deuterated butyltins (d_9-MBT, d_{18}-DBT, and d_{27}-TBT), phenyltins (d_5-MPT, d_{10}-DPT, and d_{15}-TPT), and octyltins (d_{17}-MOcT, d_{34}-DOcT, and d_{51}-TOcT). The sample was homogenized with 50 ml of 1 M HBr dissolved in ethylacetate and methanol solution (1:1). After centrifugation, the extracts in the vials were transformed to decantation balloons. NaBr saturated water and ethylacetate/hexane (3/2) were added into the balloons and shaken and then stabilized to separate the aqueous and hexane layers.

The aqueous layer was discarded and hexane layer was dehydrated with anhydrous Na_2SO_4. The moisture-free solvents were concentrated to about 1 ml by evaporator. 1 M acetate buffer, 5% sodium tetraethylborate ($NaBEt_4$) and hexane washed water were added to the solvents for ethylation. After ethylation, 1 M KOH was added for saponification of lipids in the extract. Then, hexane washed water and hexane were added to the extracts, and shaken. After saponification, hexane was added again to the water phase and extracted as described above. Anhydrous Na_2SO_4 was added to the re-extracted hexane for dehydration. The moisture-free solvent was concentrated to about 1 ml in a rotary evaporator. Concentrated solutions were cleaned up by SEP-PAK® florisil column and eluted with 5% diethylether/hexane. The final solution was concentrated under a gentle nitrogen flux to 1 ml, spiked with d_{36}-TeBT. The quantification was made using a gas chromatograph–mass spectrometer (GC/MS) (Hewlett-Packard 6870 GC system with 5973 mass selective detector and 7683 series auto sampler). GC/MS was equipped with a fused silica capillary column consisting of 0.25 mm i.d. $\times$30 m DB-1 column (100% dimethylpolysiloxane, 0.25 μm) and operated in electron impact and selected ion monitoring mode (SIM). Standard solutions of calibration curve for MBT, DBT, TBT, DPT, TPT, MOcT, DOcT, and TOcT were made at four levels of concentrations (5, 10, 50, and 250 ng/ml plus internal standard at 50 ng/ml).

Recoveries of internal standards through the whole analytical procedure were estimated based on the peak areas of internal and recovery standards. Recovery rates of internal standards spiked in samples were: $16.5 \pm 6.7\%$ (d_9-MBT), $78.6 \pm 6.2\%$ (d_{18}-DBT), $92.9 \pm 9.2\%$ (d_{27}-TBT), $59.5 \pm 15.9\%$ (d_{10}-DPT), $135 \pm 18.4\%$ (d_{15}-TPT), $56.2 \pm 11.5\%$ (d_{17}-MOcT), $103 \pm 11.0\%$ (d_{34}-DOcT), and $110 \pm 19.5\%$ (d_{51}-TOcT). Low recovery of MBT means that the concentrations of this compound must be considered semi-quantitative. Further, we did not quantify the concentrations of MPT in this study because of the poor recoveries of MPT internal standard. Reported concentration of each OT compound in the liver samples was given as nanograms of corresponding cation per gram on a wet weight basis.

The analytical procedure for total tin ($\sum$Sn) was based on the method described elsewhere (Kannan et al., 2006). In brief, liver samples were freeze–dried and then homogenized into powder. About 0.1 g of the powdered sample was weighed in a polytetrafluoroethylene (PTFE) tube and digested with concentrated nitric acid (2 ml) in a microwave oven. Concentration of $\sum$Sn was determined using an inductively coupled plasma mass spectrometry (ICP–MS) (Hewlett-Packard, HP 4500).

2.3. Quality assurance and quality control (QA/QC)

A certified reference material of fish tissue sample (NIES CRM No. 11) was analyzed by the above method. The

certified CRM concentration for TBT is 1.3 ± 0.1 μg/g and the reference value for TPT is 6.3 μg/g. Concentrations of TBT and TPT in the CRM analyzed by our method were 1.2 ± 0.3 μg/g and 6.3 ± 0.04 μg/g ($n = 3$), respectively. The recovery rates of internal standards, d_{27}-TBT and d_{15}-TPT, were $87 \pm 2.7\%$ and $106 \pm 11.3\%$, respectively. In addition, a procedural blank was included along with each analytical batch to check for interfering compounds and to correct sample values, if necessary. The detection limits of each organotin compound were calculated based on deviation (3σ) of each peak area when the standard solutions containing low levels of native compounds (1 or 5 ng/ml) were measured by GC–MS. If a peak was detected in blank, detection limit was determined as three times the peak areas. Accuracy of the analytical method for ∑Sn was checked using a standard reference material NIES CRM No. 11 Fish Tissue provided by the National Institute for Environmental Studies (NIES), Japan, and the recovery of ∑Sn was 98%.

2.4. Statistical analysis

Statistical analysis was performed using software "Statcell for excel". The Mann–Whitney *U*-test was used to compare between groups, and Spearman's correlation coefficient by rank test was used to examine the temporal trend. Simple regression analysis was applied to estimate the half-life of OTs in sea otter. A value of $p < 0.05$ was considered as significant.

3. Results and discussion

3.1. Contamination status

OT compounds were detected in all the liver samples analyzed (Table 1). Total concentrations of OTs in sea otters from California ranged from 34 to 4100 ng/g on a wet weight basis. The concentrations of OTs decreased in the following order: total butyltins (∑BTs) ≫ total octyltins (∑OcTs) ≧ total phenyltins (∑PTs). Higher concentrations of BTs than OcTs may reflect the large amount of BTs usage in the aquatic environment relative to that of OcTs, which are mainly used in terrestrial environments. Concentrations of PTs in sea otters were relatively low, which is possibly due to the limited use of TPT as an antifouling agent for boats and fishing nets in the USA (Kent, 1996). Among butyltin compounds, DBT and TBT were the predominant compounds in livers of sea otter. Higher proportions of DBT and TBT than MBT indicate that California coastal waters have been exposed to TBT until recently and/or sea otters have low metabolic capacity to degrade TBT.

3.2. Comparison of BT concentrations between 'infectious' and 'non-infectious'

Butyltin concentrations in southern sea otters in the 'infected' and 'uninfected' mortality categories were compared to understand the relationship between OTs and infectious diseases. Kannan et al. (1998) found a significant difference in BT concentrations between 'infected' and 'uninfected' mortalities from the same population earlier. In the present study, although no significant difference was found between these two groups, the average BT concentrations in infected category were consistently higher than those in the 'uninfected' group. Average BT concentrations in 'infected' mortalities were 3–4 folds higher than those in 'uninfected' mortalities, and elevated concentrations of BTs were found in 'infected' samples. The frequency distributions of the concentrations of ∑BTs between otters in 'infected' and 'uninfected' mortality categories are shown in Fig. 2. It is probable that the small size and biological variations in concentrations did not reveal the statistical significance between the two groups. Further epidemiological studies with larger number of specimens

Table 1
Mean and range of concentrations of organotins (ng/g wet weight) and total tin (μg/g dry weight; Kannan et al., 2006) in the liver of sea otters from various locations

	California (infectious diseased) $n = 15$		California (non-infectious) $n = 13$		Kamchatka, Russia $n = 5$		Alaska $n = 4$		Washington State $n = 3$	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
MBT	73	(1.9–610)	24	(5–70)	4.8	(2.7–9.9)	12	(2.1–28)	15.0	(6.8–27)
DBT	340	(16–2400)	96	(4–350)	23	(7.6–59)	70	(2.4–170)	120	(84–180)
TBT	260	(12–1300)	110	(10–390)	7.1	(2.0–17)	51	(<0.33–76)	71	(46–110)
∑BTs	680	(33–4100)	230	(21–790)	35	(18–79)	120	(6.5–270)	210	(160–250)
DPT	1.9	(<0.09–4.6)	1.7	(<0.09–5.8)	<0.09	(<0.09–0.39)	<0.09	–	<0.09	–
TPT	<0.10	(<0.10–0.89)	<0.10	–	<0.10	–	<0.10	–	<0.10	–
∑PTs	1.4	(<0.19–4.6)	0.98	(<0.19–5.8)	<0.19	(<0.19–0.39)	<0.19	–	<0.19	–
MOcT	0.51	(<0.26–1.85)	0.71	(0.34–2.3)	0.9	(<0.26–1.5)	<0.26	–	<0.26	–
DOcT	<0.09	(<0.09–0.49)	2.2	(<0.09–6.1)	<0.09	–	<0.09	–	<0.09	–
TOcT	<0.56	–	<0.56	–	<0.56	–	<0.56	–	<0.56	–
∑OcTs	0.60	(<0.91–1.8)	1.5	(0.34–6.79)	0.35	(<0.91–1.5)	<0.91	–	<0.91	–
∑OTs	680	(34–4100)	240	(21–790)	36	(18–80)	120	(6–270)	210	(160–250)
∑Sn	1.3	(0.088–6.0)	0.29	(0.077–0.86)	0.61	(0.49–0.76)	0.41	(0.036–0.86)	0.14	(0.059–0.27)

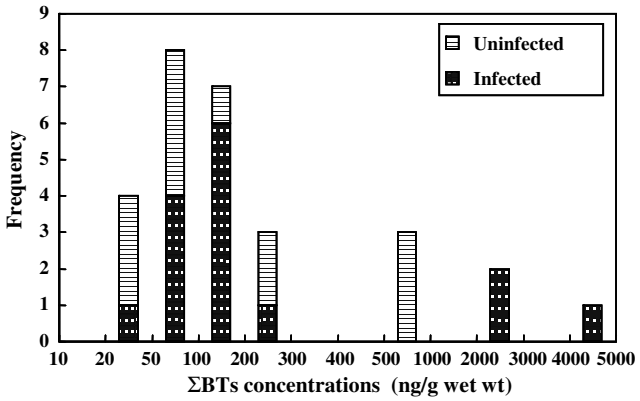

Fig. 2. Frequency distribution of ∑BTs in the liver of sea otters in 'infected' and 'non-infected' mortality categories.

are necessary to conclude the association of BTs with diseases in sea otters.

3.3. Temporal trends in BT concentrations

For understanding the temporal trends in BTs in California coastal waters, concentrations in southern sea otters collected at three locations along the California coast during 1992–2002 were examined. In addition, we combined data from this study with that reported previously by our research group (Kannan et al., 1998) to examine long-term trends of BTs in sea otters. BT (MBT, DBT, TBT and ∑BTs) concentrations in the livers of the sea otters decreased significantly since the 1990s (Fig. 3), which suggests that contamination by BTs has decreased along the California coast, reflecting the regulation of usage of TBT since 1988 in the USA. Regression analysis of ∑BTs and TBT concentrations in southern sea otters collected between 1992 and 2002 was performed to estimate the half-lives of these compounds (Fig. 4) in liver of sea otters. The analyses showed the following relationship,

$$Y_1 = -0.10(X - 1992) + 2.4 (R^2 = 0.228, p < 0.001),$$

$$Y_2 = -0.096(X - 1992) + 2.8 (R^2 = 0.236, p < 0.001)$$

where X is the sampling year of sea otter, Y_1 is log-transformed hepatic concentration of TBT and Y_2 is log-transformed hepatic concentration of ∑BTs (ng/g wet weight) in sea otter. These results indicate that hepatic concentrations of TBT, and ∑BTs in sea otters from California decreased with a half-life of 3.02 years and 3.15 years, respectively. These half-lives are longer than that found for *Caprella* spp. in Otsuchi Bay, Japan, for which the half-life of TBT and ∑BTs were 2.07 and 2.38 years, respectively, during 1994–2001 (Takeuchi et al., 2004). The relatively slow decline of TBT and ∑BTs in the liver of sea otters, compared with *Caprella* spp. from Japan, implies that TBT exposure continues even after the regulation of this compound in California and/or TBT persists in sea otter tissues for a long period due to their specific ecological characteristics.

3.4. Spatial difference

The southern sea otter's present-day range along the California coast was divided into three categories; north of Seaside, Seaside–Cayucos, and south of Cayucos (Fig. 1). The three categories were chosen based on past studies (Estes et al., 2003) and include two categories with dense human populations and one with relatively sparse inhabitation. Mean concentration of ∑OTs in sea otters

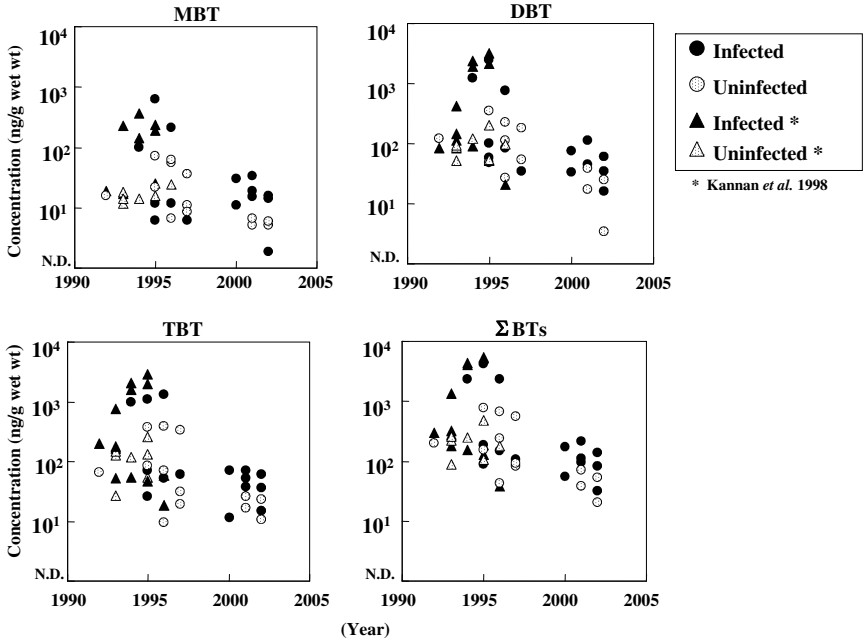

Fig. 3. Temporal trends of BTs concentrations in the liver of sea otters from California.

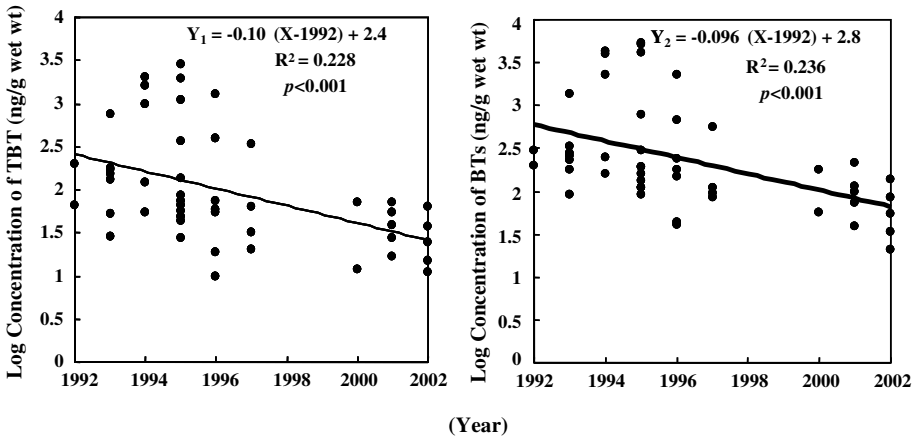

Fig. 4. Regression analysis of TBT and total butyltin (∑BTs) concentrations in sea otters collected along the California coast from 1992 to 2002.

from the three categories (north of Seaside, Seaside–Cayucos, and south of Cayucos) were 84 ng/g (range: 21–220), 710 ng/g (range: 45–4100), and 120 ng/g, wet weight (range: 41–200), respectively. OT concentrations were significantly higher ($p < 0.05$) in the central segment (Seaside–Cayucos) than in the north of Seaside segment. OTs concentrations were not significantly different between the south of Cayucos and the central segment ($p > 0.05$). Theses results suggest higher contamination by OTs along the central California coast. Interestingly, sea otters found dead along the California coast contained higher concentrations of BTs than those from other locations such as Washington and Alaska in the USA and Kamchatka in Russia. Especially, a significant difference was found in BTs concentration between California sea otters and sea otters found dead along Kamchatka (Fig. 5). In addition, the composition of TBT to ∑BTs in southern sea otter livers analyzed in this study was similar to that reported in a previous study (Kannan et al., 1998) (Fig. 6). This suggests that TBT exposure in California sea otters continues, although the amount of exposure may be decreasing (Fig. 3). Moreover, the percentage of TBT to ∑BTs was similar among sea otters from all the areas studied. This may indicate fresh input of TBT originating from human activities not only in California but also in other areas studied.

3.5. Comparison with other marine mammal species

BTs concentrations in the liver of southern sea otters were compared with those of pinnipeds including northern elephant seal (*Mirounga augustirostris*), California sea lion (*Zalophus californianus*), and harbor seal (*Phoca vitulina*) found dead along California coastal waters during 1991–1997 (Kajiwara et al., 2001). Sea otters from the California coast contained higher concentrations of BTs than pinnipeds stranded along the California coast, and BT concentrations in sea otters were significantly greater than in California sea lions and harbor seals ($p < 0.05$) (Kajiwara et al., 2001). In addition, the proportion of TBT to total BTs was greater in sea otters than in pinnipeds. Theses differences may be explained by ecological and physiological

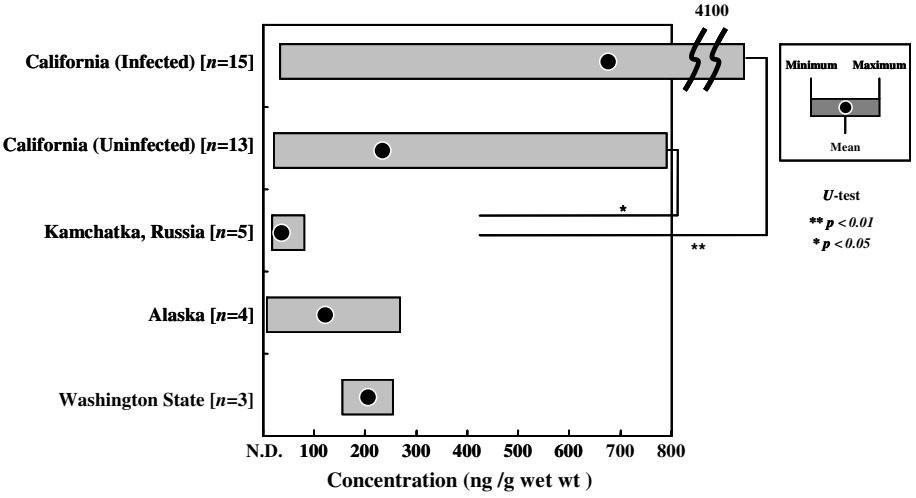

Fig. 5. Comparison of BT concentrations in the livers of sea otters from California, Washington, Alaska (USA), and Kamchatka (Russia). N.D. means not detected.

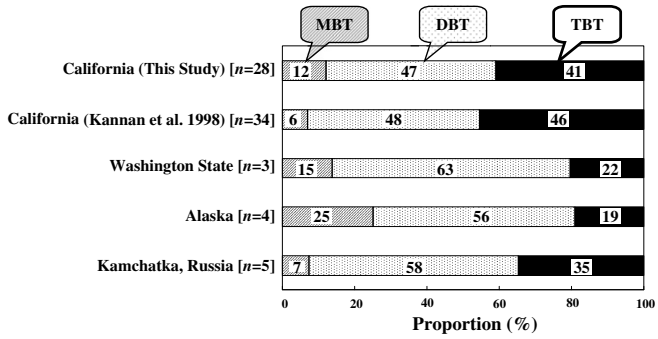

Fig. 6. Composition of BTs in the livers of sea otters from California, Washington, Alaska (USA), and Kamchatka (Russia) (this study) and data from a previous study of California sea otter livers (Kannan et al., 1998).

factors. First, the prey of sea otters and pinnipeds differ, so that OT exposure through the food chain would differ. Secondly, sea otters consume large amounts of food and thus intake of TBT from food may be higher than that for pinnipeds. Finally, sea otters may have a low metabolic capacity to degrade OTs into an inorganic form. These factors may lead to higher accumulation of BTs and higher proportion of TBT in sea otters than in pinnipeds. Some enzyme profiles have been shown to differ between sea otters and other marine mammals. A previous study estimated activities of phenobarbital (PB)-type and methyl-cholanthrene (MC)-type enzymes in sea otters based on the profiles polychlorinated biphenyl congener (Kannan et al., 2004). Based on the congener profiles, the activities of both MC- and PB-type enzymes in sea otters were relatively higher than in other marine mammal species (Kannan et al., 2004). Measurements using fresh livers are needed to reveal drug metabolizing enzyme activities and to clarify the activities of enzymes that metabolize OTs. No study has reported the enzymes involved in OT metabolism in marine mammals and further studies are need in this regard.

3.6. Percentage of organotins in total tin concentrations

For elucidating the metabolic capacity of sea otters to degrade OTs into inorganic tin, the percentage of OTs in total tin ($\sum$Sn) concentrations in sea otters collected from various areas were compared with those of pinnipeds such as Steller sea lion and northern fur seal, and cetaceans including bottlenose dolphin and finless porpoise (Fig. 7). In sea otters, the proportion of BTs in $\sum$Sn concentrations was higher in specimens found dead along the California coast than in those found dead in other areas. This may reflect differences in exposures to OTs from human activities in each area. The percentage of BTs (average 79%) in $\sum$Sn in sea otters was comparable to those of cetaceans, but significantly higher than those in pinnipeds. Cetaceans are well known as sensitive animals to chemical contamination because of the low cytochrome P-450 activity (Tanabe, 2002). Based on this comparison, it can be suggested that sea otters may have similar lower metabolic capacity to degrade OTs into inorganic forms.

3.7. Risk assessment

Concentrations of TBT and DBT in sea otters were compared with the thresholds for immunotoxicity and hepatotoxicity (Nakata et al., 2002; Ueno et al., 1994) (Table 1). Nakata et al. (Nakata et al., 2002) exposed peripheral blood mononuclear cells (PBMCs) isolated from Dall's porpoises (*Phocoenoides dalli*) to varying concentrations of BTs and demonstrated that cocanavalin A – stimulated mitogenesis is significantly suppressed ($p < 0.01$) when the cells were exposed at 89 ng/ml of TBT and 77 ng/ml of DBT. The corresponding EC_{50} of TBT and DBT in blood was 44 and 33 ng/g wet weight. These values were converted into hepatic concentrations using the concentration ratio between blood and liver (1:25) (Takahashi and Tanabe, 2007). In present study concentrations of TBT and

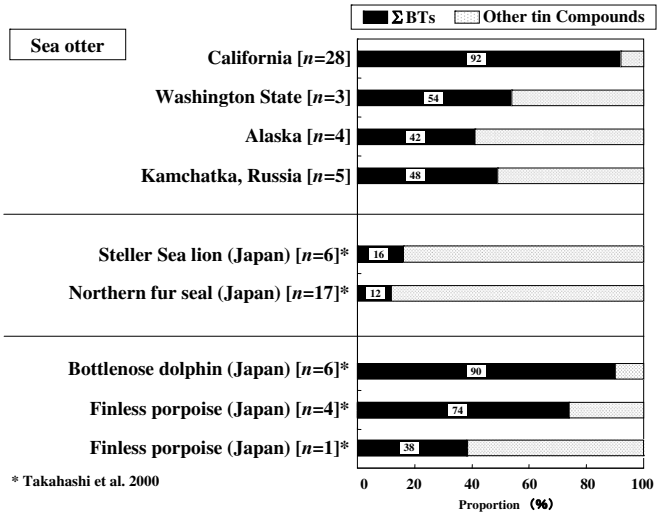

Fig. 7. Percentage of $\sum$BTs (normalized Sn ng/g dry weight) in $\sum$Sn concentrations in the livers of sea otters and other marine mammals. See above mentioned reference for further information.

DBT found in sea otters in the 'infected' mortality category ranged from 16 to 2400 ng/g wet weight for DBT and 12 to 1300 ng/g wet weight for TBT, and the concentrations in a few infected sea otters were close to or above the threshold levels of immunotoxicity and hepatotoxicity in other species (Kannan et al., 1997; Nakata et al., 2002; Ueno et al., 1994). This result implies that OTs, particularly BTs, pose high risk to the health of sea otters. No in vivo and in vitro studies reported the immunotoxic and hepatotoxic effects by OTs on mustelids such as otters. Animals of this family accumulate high concentrations of PCBs (Kannan et al., 2004; Lopez-Martin and Ruiz-Olmo, 1996; Smit et al., 1998; Simpson et al., 2000; Nakata et al., 1998), and are known to be sensitive to chemical contamination. Further studies with chronic, low level BT exposures and critical effects in sea otters are needed for accurate risk assessment.

This study presents for the first time TBT and $\sum$BTs half-lives in southern sea otters, and indicates the temporal trend in BTs in otters found dead along the California coast after the regulation of the usage of TBT in the USA. Furthermore, occurrence of OcTs and PTs along the California coast is reported for the first time. The study shows that the California coast is affected by BT contamination more than OcTs and PTs. Concentrations of BTs in southern sea otters are much higher than that in pinnipeds found dead along the California coast. Although OT concentrations in sea otters were decreasing since the 1990s, continuing high adult mortality with infectious disease is a concern. Recent studies have shown that a few contaminant classes are elevated in sea otters dying from infectious rather than non-infectious causes suggesting the role of contaminants in diseases of sea otters (Kannan et al., 2006a; Kannan et al., 2006b). It is possible that exposure to multiple contaminations in concert affect the health of sea otters. Further studies are needed to examine the cause-effect linkage and other factors that contribute to the high rates of infectious diseases in sea otters.

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