Environ. Sci. Technol. **1998**, *32,* 1169−1175

Butyltin Residues in Southern Sea Otters (*Enhydra lutris nereis*) Found Dead along California Coastal Waters

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Tributyltin (TBT) and its degradation products, mono- (MBT) and dibutyltin (DBT), were determined in liver, kidney, and brain tissues of adult southern sea otters (*Enhydra lutris nereis*) found dead along the coast of California during 1992−1996. Hepatic concentrations of butyltin compounds (BTs = MBT + DBT + TBT) ranged from 40 to 9200 ng/g wet wt, which varied depending on the sampling location and gender. Concentrations of BTs in sea otters were comparable to those reported in stranded bottlenose dolphins from the U.S. Atlantic Coast during 1989−1994. Greater accumulation of butyltins in sea otters was explained by their bottom-feeding habit and the diet that consists exclusively of invertebrates such as mollusks and gastropods. Livers of female sea otters contained approximately 2-fold greater concentrations of BTs than did those of males. The composition of butyltin compounds in sea otter tissues was predominated by TBT in most cases and suggestive of recent exposure. Large harbors such as Monterey Harbor that handle ships legally painted with TBT-containing antifouling paints continued to experience ecotoxicologically significant butyltin contamination. Sea otters, which were affected by infectious diseases, contained greater concentrations of BTs in their tissues than those that died from trauma and other unknown causes.

Introduction

Tributyltin (TBT) has been used extensively since the 1960s as an antifouling agent in marine paint formulations to prevent the attachment of barnacles and slime on boat hulls and aquaculture nets (*1*). TBT ablated from hulls and seapen nets could migrate into water, sediment, and biota. It became evident in the early 1980s that TBT leached from these paints affected organisms other than those targeted. Concern for the environmental impact of TBT and its degradation products, dibutyltin (DBT) and monobutyltin (MBT), grew after the deleterious effects on reproduction in Pacific oyster (*Crassostrea gigas*) were reported (*2*). Similarly, in the eastern United States, the imposition of male sex characters (imposex) on female mud snails (*Ilyanassa obsoleta*) was linked to TBT leached from antifouling paints (*3*). Experimental and field observations have shown that some mollusks are sensitive to seawater TBT levels below 10 ng/L (*4*). Deleterious effects of TBT have been observed in microalgae, bivalve mollusks, polychaetes, crustaceans, and fish at concentrations as low as 10−100 ng/L (*4*). As a consequence, regulations on the use of TBT-based antifouling paints have been introduced in several countries since the late 1980s (*1*). Even though a decrease in the TBT contamination was recorded after regulations were introduced, concentrations persisted at levels considered to be chronically toxic for most susceptible organisms in areas frequented by large vessels (*5*−*8*). In addition, TBT persists in sediments for several years and continues to pose a potential threat to aquatic organisms.

While a great deal of attention has been paid to accumulation and toxic effects of butyltin compounds in lower trophic aquatic organisms such as bivalve mollusks and gastropods, studies in higher trophic vertebrate predators were not available until 1995. Only recently has significant accumulation of butyltin compounds in marine mammals (*9*−*14*) and fish-eating water birds (*15*−*17*) been demonstrated.

TBT and DBT have well-documented immunosuppressing potential in fish and mammals (*18, 19*). Greater accumulation of these compounds in addition to several other factors may impair immunocompetence in animals and increase susceptibility to microbial infections. Exposure of fish and several marine invertebrates at environmental levels of TBT significantly affected the phagocytic activity and decreased the resistance against pathogenic microorganisms (*20*−*22*). Although establishment of such an association between butyltin accumulation and disease in animals in the wild could be confounded by several variables such as nutritive status, genetic vulnerability, and exposure to other contaminants, studies that describe accumulation features of immunosuppressing agents are necessary to assess environmental risks and to regulate their use.

Otters, which belong to the family Mustelidae, are fish-eating aquatic mammals and are sensitive to chemical contamination. Studies have linked polychlorinated biphenyl (PCB) contamination in tissues to pathological conditions and population decline in European otters, *Lutra lutra* (*23*). Similarly, recent studies have shown the prevalence of infectious diseases in southern sea otters (*Enhydra lutris nereis*) found dead in California coastal waters, which raised concern about their immune function and health of the environment (*24*). Despite this, little is known about contaminant accumulation in southern sea otters. Sea otters provide good indicators of marine pollution because they are nonmigratory. Contaminant burdens in sea otters should reflect their local habitats in combination with metabolic factors. In this study, tissues of adult sea otters found dead in California coastal waters were analyzed for the presence of butyltin compounds. Accumulation of butyltin compounds by sea otters and its relation to otter health were also examined.

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S0013-936X(97)00914-0 CCC: $15.00
Published on Web 03/13/1998

Materials and Methods

Samples. Sea otters that died along the coast of California during 1992−1996 were collected through a stranding network coordinated by the U.S. Fish and Wildlife Service and the California Department of Fish and Game with the cooperation of other federal agencies, academic, and private institutions. Otter carcasses in good postmortem condition were rapidly chilled or frozen and shipped overnight to the National Wildlife Health Center (Madison, WI) for necropsy. Postmortem examinations were performed to determine the causes of death. A variety of tissues from most animals were fixed in 10% buffered formalin for histopathology, then paraffin embedded, stained by hematoxylin and eosin, and examined by light microscopy. Selection of other diagnostic laboratory tests was based on the history and gross lesions and included various microbiologic, virologic, and parasitologic procedures. The major cause of mortality was identified to be infectious diseases (*24*).

In general, the deaths involved only a single animal. However, there were two events in which multiple animals were found dead in Monterey Harbor in July 1995 and in Estero Bay in July 1996. Two otters from the Monterey Harbor mortality event (samples 13760 and 13763 of Table 1) and two otters from the Estero Bay event (samples 14381 and 14387, Table 1) were available for this study. Total length (tip of the nose to the tip of the tail) and body weight were measured prior to necropsy.

The brain, liver, and kidney were collected from the carcasses at the time of necropsy, wrapped in aluminum foil or whirlpac bags, and stored frozen at −20 °C until analysis. Thirty-five adult animals comprising 18 males and 17 females were selected for this study. Liver tissue from each otter, and in addition, kidney samples from 10 otters and brain samples from five otters were analyzed. Animal identification number, collection date and location, weight, length, gender, and cause of death are given in Table 1, and sampling locations are given in Figure 1. The otters in this group died from infectious diseases ($n = 14$), trauma (*9*), unknown causes (*7*), or miscellaneous problems (*5*). On the basis of the postmortem diagnostics, the nine otters that died from trauma were generally healthy. The traumatic injuries were acutely fatal (shark bite, 5; gunshot, 3; fractured skull, 1), and they did not have apparent health problems that predisposed them to trauma. Among several sampling locations (Figure 1), Half Moon Bay, Moss Landing, Monterey Harbor, Estero Bay, Morro Bay, and Port San Luis have fishing and pleasure boating activities; Carmel and San Simeon are pristine, unpolluted areas; Montara Beach, Hazard Canyon, Diablo Canyon, Pismo Beach and Dunes, and Coal Oil Point are more open areas with less intensive boat traffic.

Exact age determination has not been well-established for California sea otters. The adult age designation was based on dentition and total length measurements (*25*). Generally, adults have no deciduous teeth and indications of at least early wear on their permanent teeth. Adult females are those >105 cm in total length (about 4−5 yr of age or more), and males are >115 cm (about 5 yr or more). The life span of California sea otters is not well-documented but thought to be about 12−16 yr in the wild (*26*).

Chemical Analysis. The analytical method used for the determination of MBT, DBT, and TBT in otter tissues has been described elsewhere (*9, 27*). Briefly, acidified tissue samples were homogenized with 70 mL of 0.1% tropolone−acetone, and the solvent was transferred to 100 mL of 0.1% tropolone−benzene. Moisture in the organic extract was removed using 35 g of anhydrous sodium sulfate, and then the concentrated extract was propylated with *n*-propylmagnesium bromide (ca. 2 mol/L in THF solution, Tokyo Kasei Kogyo Ltd., Japan) as a Grignard reagent. The derivatized extract was passed through a 6-g Florisil packed wet column for cleanup. The eluate from the Florisil column was rotary evaporated to 5 mL and injected into a gas chromatograph.

For the quantification of butyltin compounds, a capillary gas chromatograph with a flame photometric detector (GC−FPD) was used. Chromatographic separation was performed on a Hewlett-Packard 5890 series II gas chromatograph with a 30 m × 0.25 mm (i.d.) DB-1 capillary column coated at 0.25 μm film thickness. The column oven temperature was programmed from 80 °C (1-min hold) to 160 °C at a rate of 15 °C/min and then at a rate of 5 °C/min to a final temperature of 260 °C with a 5-min final hold time. Injector and detector temperatures were held at 200 and 270 °C, respectively. The flame photometer was operated using a hydrogen−air−nitrogen flame and was equipped with a 610 nm band-pass filter that is selective for tin-containing compounds.

Butyltin trichloride, dibutyltin dichloride, and tributyltin chloride of known amounts (100 ng) were spiked into the liver of Antarctic minke whale (*Balenoptera acutorostrata*), containing butyltins below the limit of detection, passed through the whole analytical procedure, and used as an external standard. Only freshly derivatized external standards prepared along with every set of eight samples were used to estimate concentrations. Concentrations were quantified by comparing peak heights of butyltins in samples with those in the external standards. Tributylhexyltin (synthesized by the reaction of *n*-hexylmagnesium bromide with tributyltin chloride) was added to each sample as an internal standard prior to extraction. A procedural blank was analyzed with every set of eight samples to check for interfering compounds and to correct sample values, if necessary. Monobutyltin, probably originating from commercial solvents or reagents that came into contact with PVC containing this compound as a stabilizer, was found at trace levels in reagent blanks. The values obtained for MBT in samples were therefore corrected for blank concentrations. However, blanks never contained TBT. The detection limits of MBT, DBT, and TBT were 7, 2.4, and 0.5 ng/g wet wt, respectively. The average recovery rates of monobutyltin trichloride, dibutyltin dichloride, and tributyltin chloride dissolved in hexane, spiked into the sample matrix, and passed through the whole analytical procedure were between 90 and 110% for each compounds. All concentrations reported in this study refer to butyltin species as the corresponding ion, and they were not corrected for the recovery of the internal standard (which was between 85 and 105%). Statistical differences were determined at the 95% confidence interval for several of the comparisons using the Student's *t*-test.

Results and Discussion

Mean hepatic concentration of BTs (MBT + DBT + TBT) in sea otters was 1320 ± 2050 ng/g wet wt ($n = 35$). Hepatic concentrations of BTs ranged from 40 to 9200 ng/g wet wt (Table 1). The greatest concentration of 9200 ng/g found in the liver of a sea otter was slightly less than that found in a diseased bottlenose dolphin (*Tursiops truncatus*) collected from the U.S. Atlantic Coast (11340 ng/g wet wt) in 1989 (*12*) and in a dead finless porpoise (*Neophocaena phocaenoides*) collected from Japanese coastal waters (10 200 ng/g wet wt) in 1985 (*9*). The value of 9200 is 4.5 times the standard deviation and appears to qualify statistically as an outlier (*28*). Therefore, this sample value was removed from further discussions. Nevertheless, repeated analysis of this sample tissue yielded similar values for total butyltins, which suggested that a maximum concentration of at least 9200 ng/g could be expected in sea otter populations. Further studies with greater numbers of samples are needed to examine the reason for such anomalously elevated concentrations in individual sea otters. When the outlier was removed, the mean concentration of total butyltins in the

TABLE 1. Concentrations (ng/g Wet Wt) of Butyltin Compounds in Liver, Kidney, and Brain Tissues of Sea Otter Found Dead along California Coastal Waters during 1992−1996

sample ID	sex	date collected	location	cause of death	length (cm)	weight (kg)	MBT	DBT	TBT	BTs[a]
				Liver						
13677-001	m	15-Apr-95	Moss Landing	trauma	ND[b]	25.3	360	5820	3020	9200
13773-001	m	24-Jul-95	Moss Landing	disease	127	17.6	25	53	48	130
13791-001	m	30-Jul-95	Morro Bay	disease	139	29.4	236	3090	1970	5300
14373-001	m	11-Jul-96	Morro Bay	disease	123	20.3	<7	21	19	40
10901-001	m	29-Mar-92	Morro Bay	intestinal perforation	129	31.5	81	860	2130	3070
13227-001	m	3-Dec-94	Morro Bay	esophageal impaction	130	21.7	10	104	100	210
13318-001	m	11-Jan-95	Half Moon Bay	trauma	127	23	<7	51	56	110
13712-001	m	26-Jun-95	San Simeon	trauma	128	29.3	<7	54	135	190
11559-001	m	2-May-93	Montara Beach	trauma	129	27.1	18	85	130	230
11450-001	m	9-Apr-93	Pismo Beach	disease	134	25.3	14	83	180	280
10696-002	m	7-Apr-92	Pismo Dunes	disease	124	24.5	19	83	200	300
11514-001	m	6-May-93	Jalama Beach	disease	137	25.2	220	410	760	1390
13110-001	m	3-Oct-94	Coal Oil Point	trauma	127	26.2	14	120	120	250
14387-001	m	25-Jul-96	Estero Bay	undetermined	125	25.5	<7	49	75	120
14381-001	m	20-Jul-96	Estero Bay	undetermined	125	23.1	<7	29	32	61
11937-001	m	18-Nov-93	Estero Bay	trauma	126	18	12	52	28	92
11740-001	m	16-Aug-93	Estero Bay	trauma	124	30.5	14	94	150	260
11336-001	f	11-Feb-93	Estero Bay	disease	120	14.8	17	144	170	330
11631-001	f	27-Jun-93	Estero Bay	disease	117	19.7	18	110	53	180
11510-001	f	5-May-95	Estero Bay	undetermined	123	16.4	31	110	53	190
11019-001	f	5-Sep-92	Estero Bay	Emaciation	108	15	26	220	140	390
12526-001	f	12-Jan-94	Monterey Harbor	disease	126	14.8	360	2360	1600	4320
13316-001	f	8-Jan-95	Monterey Harbor	disease	116	13.7	190	2080	2900	5170
11993-001	f	31-Dec-93	Monterey Harbor	urinary obstruction	118	20.2	140	1520	950	2610
11538-001	f	15-May-93	Monterey Harbor	undetermined	122	13.1	17	220	190	430
13219-001	f	23-Nov-94	Monterey Harbor	disease	123	19.4	140	1840	2050	4030
13760-001	f	20-Jul-95	Monterey Harbor	undetermined	125	18.9	310	1560	670	2540
13763-001	f	22-Jul-95	Monterey Harbor	undetermined	124	16.4	12	310	330	650
11248-001	f	14-Jan-93	Monterey Harbor	neoplasia	118	18.8	140	1290	920	2350
12577-001	f	6-Feb-94	Hazard Canyon	disease	119	16.3	14	90	55	160
13479-001	f	20-Mar-95	Diablo Canyon	disease	124	16.6	18	240	43	300
14063-001	f	21-Dec-95	Diablo Canyon	disease	123	23.5	9.4	34	66	110
11380-001	f	3-Mar-93	Port San Luis/Avila	undetermined	115	14.9	8	120	60	190
14158-001	f	22-Mar-96	Carmel	trauma	120	19.4	24	95	59	180
13425-001	m	23-Feb-95	Carmel	trauma	124	22.7	16	200	260	480
				Kidney						
13677-001	m	15-Apr-95	Moss Landing	trauma	ND	25.3	11	54	200	265
13791-001	m	30-Jul-95	Morro Bay	disease	139	29.4	17	200	210	430
14373-001	m	11-Jul-96	Morro Bay	disease	123	20.3	<7	<2.4	4	4
10901-001	m	29-Mar-92	Morro Bay	intestinal perforation	129	31.5	7	24	120	150
13318-001	m	11-Jan-95	Half Moon Bay	trauma	127	23	<7	3.7	10	14
13712-001	m	26-Jun-95	San Simeon	trauma	128	29.3	<7	7.5	7.7	15
14387-001	m	25-Jul-96	Estero Bay	undetermined	125	25.5	14	6.8	9.3	30
11019-001	f	5-Sep-92	Estero Bay	emaciation	108	15	<7	15	16	31
12526-001	f	12-Jan-94	Monterey Harbor	disease	126	14.8	61	77	75	210
13316-001	f	8-Jan-95	Monterey Harbor	disease	116	13.7	11	110	170	290
				Brain						
13677-001	m	15-Apr-95	Moss Landing	trauma	ND	25.3	12	24	105	140
13791-001	m	30-Jul-95	Morro Bay	disease	139	29.4	<7	9.3	72	81
13318-001	m	11-Jan-95	Half Moon Bay	trauma	127	23	<7	<2.4	3.9	3.9
13712-001	m	26-Jun-95	San Simeon	trauma	128	29.3	<7	<2.4	2.7	2.7
14387-001	m	25-Jul-96	Estero Bay	undetermined	125	25.5	<7	2.8	1.8	4.6

[a] BTs = MBT + DBT + TBT. [b] ND, not determined.

liver was 1090 ± 1560 ng/g wet wt (n = 34). The mean hepatic concentrations of BTs in sea otters were similar to those reported for bottlenose dolphins (1400 ng/g wet wt) found stranded along the U.S. Atlantic Coast (*12*). In general, butyltin concentrations in sea otters were in the greater range of those values reported so far for various marine mammals (*9*−*14*, *29*).

Total butyltin concentrations in kidney and brain were in the ranges of 4−430 (mean: 160 ± 140) and 2.7−140 (mean: 61 ± 56) ng/g wet wt, respectively, which were about 7- and 18-fold less than those in the liver. The liver-to-kidney ratios of butyltin concentrations in sea otters were at least 2-fold greater than those in cetaceans or pinnipeds collected off Japanese coastal waters (*10*). This may suggest the presence of specific physiological processes in sea otters that favor greater accumulation in liver and/or rapid elimination from kidney. The liver and kidney of sea otters were large in proportion to body size, accounting for about 5.7 and 2% of body weight, respectively (*26*). Great concentrations of butyltins in such large livers suggest the presence of great

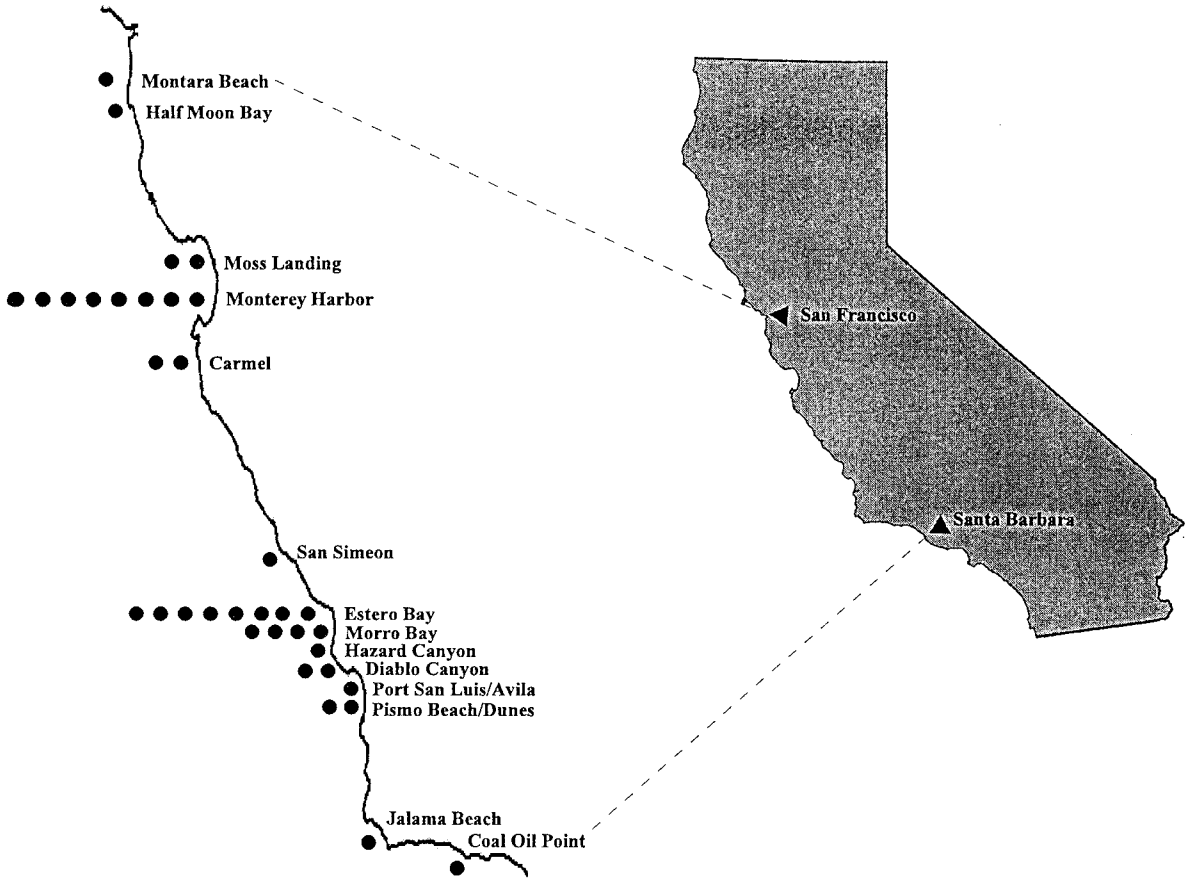

FIGURE 1. Map showing sampling locations of sea otters in California coastal waters.

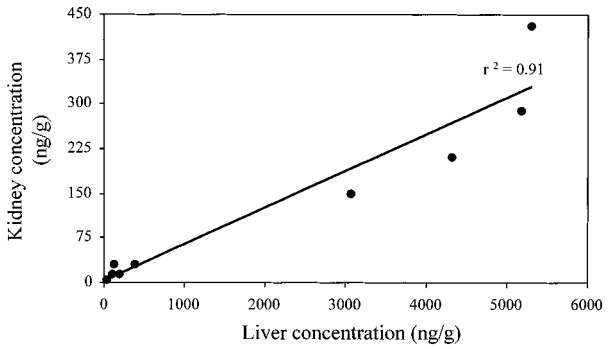

FIGURE 2. Relationship of butyltin concentrations in liver with those of kidney in southern sea otters.

burdens of these compounds in target organs. On the basis of the mean body weight of sea otters analyzed (21.1 kg), the average burden of BTs in the liver was estimated to be 1.3 mg. The sea otter that contained the greatest BTs concentration of 9200 ng/g had 13.3 mg of BTs in the liver. The ranges of hepatic burdens of BTs in sea otters were similar to those observed in dead finless porpoises from Japan (*9*) and diseased bottlenose dolphins from the United States (*12*). Total butyltin concentrations in the liver were positively correlated with those in brain ($r^2 = 1$, $p < 0.01$) and kidney ($r^2 = 0.91$, $p < 0.01$) (Figure 2), which is similar to that reported for bottlenose dolphins (*12*).

Despite the regulation on the use of TBT in the United States in 1988 (*30*), concentrations in sea otter were great. Greater accumulation of butyltins in the California sea otter could be explained by their bottom-feeding habits. Sea otters feed exclusively on macroinvertebrates such as mussels, abalone, rock crabs, red sea urchin, clams, turban snails, barnacle, scallops, echiuroid worms, sea star, and chitons, which usually accumulate greater levels of butyltin compounds than fin fishes. Concentrations of TBT in coastal waters of California in the late 1980s were greater than 50 ng/L, and all the Pacific oysters (*Crassostrea gigas*) examined had chambering effects and abnormal shell thickening, both of which are characteristic effects of TBT. TBT concentrations in oysters were as great as 1500 ng/g dry wt in Moss Landing and Monterey Harbor (*31*). As for sea otters, mollusk-feeding water birds were shown to accumulate greater concentrations of butyltin compounds than predatory birds, which feed on fish and mammals (*32*). In addition, because of their small body size and lack of blubber (which provides insulation as well as a reserve of stored energy in other marine mammals), sea otters compensate for thermal stress by maintaining an elevated metabolic rate (i.e., high internal heat production). Therefore, food requirements of sea otters are great, and they consume an amount of food equivalent to 23−33% of their body weight each day (*26*). Thus, great amounts of food intake, specifically benthic invertebrates from nearshore coastal areas could explain elevated accumulation of BTs in sea otters.

Mean hepatic concentrations of butyltins in female (1420 ng/g wet wt) sea otters were 2-fold greater than those in males (750 ng/g wet wt). Concentrations of BTs in kidneys of females were also greater than those in males; however, the difference was relatively less than that of the liver. The mean renal concentration of BTs in females was 190 ng/g wet wt, whereas that in males was 142 ng/g wet wt (Table 2). Even in a given geographical area, for example, in Estero Bay and Carmel, livers of females contained at least 2-fold greater concentrations of BTs than those of males. Although the gender differences in BT concentrations were not statistically significant ($p > 0.05$), greater concentrations in females may be due to their higher feeding rates and less dilution effect as a result of smaller body size than males. A

TABLE 2. Mean Concentrations (ng/g Wet Wt) of Butyltin Compounds in Liver, Kidney, and Brain Tissues of Male and Female Sea Otters from California Coastal Waters

organ/gender	MBT	DBT	TBT	BTs[a]
		Liver (n = 34)		
male	57	320	376	753
female	87	726	606	1420
		Kidney (n = 10)		
male	12	49	80	141
female	36	67	87	190
		Brain (n = 5)		
male	12	12	37	61

[a] BTs = MBT + DBT + TBT.

similar observation has been made in Risso's dolphins (*Grampus griseus*) from the Pacific Coast of Japan, in which females had 1.5-fold greater concentrations of BTs than males (*14*). Hepatic concentrations of BTs were correlated with length and weight in adult males ($p < 0.05$), whereas in females the concentrations remained steady (Figure 3).

Dibutyltin was the predominant species of butyltin compounds in female livers (51%), while TBT accounted for the major proportion in male livers (50%). Kidney (46−56%) and brain (61%) tissues also contained a greater percent of TBT than its metabolites (Figure 4, available as Supporting Information). In cetaceans, the percentage of DBT to total butyltin concentrations in livers was >65% for both sexes (*9, 12, 14*), which suggested the metabolic decomposition of TBT to DBT in the liver. A relatively lesser proportion of DBT in sea otters than in cetaceans may suggest recent exposure to TBT and/or less efficient biodegradation of TBT by sea otters than cetaceans.

Concentrations of butyltins in sea otters varied according to sampling locations (Table 3). Sea otters collected from enclosed marinas such as Monterey Harbor and Morro Bay contained at least an order of magnitude greater concentrations than those from open locations. In particular, hepatic concentrations of BTs in sea otters from Monterey Harbor were significantly greater than those from Carmel and San Simeon ($p < 0.05$) and in other open locations ($p < 0.01$). TBT constituted the major proportion of total butyltin

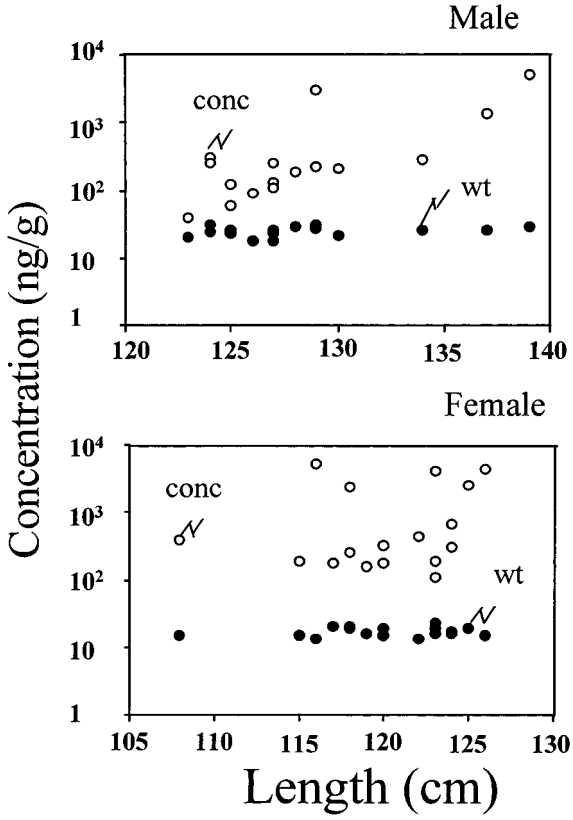

FIGURE 3. Relationship of butyltin concentrations in liver with length and weight of sea otters.

concentrations in a few otters from Monterey Harbor. This suggests that sea otters collected at areas with concentrated boating activities and enclosed marinas experience greater exposure to BTs.

A 3-yr profile of concentrations of butyltins were examined in the livers of adult female sea otters collected from Monterey Harbor (Table 4). Concentrations remained great relative to other areas, even in 1995. Values as great as 5170 ng/g wet wt were observed. Nevertheless, no specific trend in butyltin concentrations could be discerned from 1993 to 1995 ($p >$

TABLE 3. Concentrations of Butyltin Compounds (ng/g Wet Wt) Sea Otter Livers from Various Locations in Coastal California

location	sex	n	MBT	DBT	TBT	BTs[a]
Moss Landing	m	2	193 (25−360)	2940 (53−5820)	1530 (48−3020)	4670 (130−9200)
Morro Bay	m	4	84 (<7−236)	1020 (21−3090)	1060 (19−2130)	2160 (40−5300)
Half Moon Bay	m	1	<7	51	56	110
San Simeon	m	1	<7	54	135	190
Estero Bay	m	4	10 (<7−14)	56 (29−94)	71 (28−150)	130 (61−260)
	f	4	23 (17−31)	150 (110−220)	104 (53−170)	270 (180−390)
Pismo Beach	m	2	17 (14−19)	83 (83)	190 (180−200)	290 (280−300)
Monterey Harbor	f	8	160 (12−360)	1400 (220−2360)	1200 (190−2900)	2760 (430−5170)
Montara Beach	m	1	18	85	130	230
Hazard Canyon	f	1	14	90	55	160
Jalama Beach	m	1	220	410	760	1390
Diablo Canyon	f	2	14 (9.4−18)	137 (34−240)	55 (43−66)	205 (110−300)
Port San Luis/Avila	f	1	8	120	60	190
Carmel	f	1	24	95	59	180
	m	1	16	200	260	480

[a] BTs = MBT + DBT + TBT. Values in parentheses indicate the range.

TABLE 4. Trends in Concentrations of Butyltins (ng/g Wet Wt) in the Liver of Female Sea Otters Collected from Monterey Harbor during 1993−1995

year	*n*	MBT	DBT	TBT	BTs[a]
1993	3	99 (17−140)	1010 (220−1520)	690 (190−950)	1800 (430−2610)
1994	2	250 (140−360)	2100 (1840−2360)	1830 (1600−2050)	4180 (4030−4320)
1995	3	170 (12−310)	1320 (310−2080)	1300 (330−2900)	2790 (650−5170)

[a] BTs = MBT + DBT + TBT. Values in parentheses indicate the range.

TABLE 5. Total Concentrations of Butyltins (ng/g Wet Wt) in the Liver of Sea Otters in Relation to Causes of Death

cause of death	*n*	total BTs[a]
infectious disease	14	1570 (40−5300)
trauma	8[b]	220 (92−480)
unknown causes	7	600 (61−2540)
miscellaneous problems	5	1730 (210−3070)

[b] BTs = MBT + DBT + TBT. Values in parentheses indicate the range.
[b] Outlier removed.

0.05). TBT concentrations in bivalves in some locations have declined after regulations were implemented in the United States, but in other locations concentrations had not declined at the time of sampling (*33*). A recent survey conducted in Canada demonstrated that the regulation of TBT has not been effective in reducing concentrations in several locations, particularly in large harbors and coastal areas, that handle ships (>25 m long) legally painted with TBT-containing antifouling paints (*8*). Our results indicate that TBT exposure still persists at considerable levels in biota, particularly in large harbors such as Monterey Harbor, in coastal California.

Although no laboratory feeding studies of butyltins to sea otters were available to evaluate the sensitivity of these animals to butyltins, studies with PCBs exposure in closely related mustelids such as river otters and mink have shown that these animals are extremely sensitive to chemical contamination (*23*). Due to the lack of threshold effect concentrations (i.e., reference doses) and several confounding variables, establishment of relationship between TBT and immunosuppression in sea otters is difficult at this stage and needs further investigation. Since both the concentrations of BTs and the health of individuals analyzed were available, a comparison between two parameters could be made. Otters that died of infectious diseases contained BTs (mean: 1570 ng/g) greater than those that died of trauma (220 ng/g) ($p < 0.05$) (Table 5). However, the range of BT concentrations in diseased animals was great. The group of animals that died of miscellaneous problems including intestinal perforation, esophageal impaction, urinary obstruction, and neoplasia contained >2350 ng/g total BT concentrations, which were comparable to those died from infectious diseases ($p > 0.05$). Some of these problems such as urinary obstruction and intestinal perforations may have originated from bacterial infections (*24*). When only Monterey Harbor female otters were considered, those that died of infectious diseases contained, on average, 4510 ng/g wet wt total BTs in the liver, which were at least 2−4-fold greater ($p < 0.01$) than those in animals that died from urinary obstruction, neoplasia, and undetermined causes. Tissues from more healthy animals need to be examined to make further evaluations of the effect of butyltin compounds on disease in sea otters. Examination of other immunotoxic contaminants (such as PCBs and other organochlorines) is needed to evaluate their significance in disease development in sea otters. A recent report showed that the hepatic concentrations of PCBs in adult male sea otters found dead in California coastal waters during 1988−1991 were, on average, 170 ng/g wet wt (*34*), which was less than those of BTs. These results are in line with those of our previous studies, which showed elevated accumulation of BTs in diseased and/or stranded cetaceans from the U.S. (*12*) and Japanese (*9*) coastal waters.

Acknowledgments

Portions of this research was supported by a National Institute of Environmental Health Sciences (NIEHS) Superfund Basic Research Program (ES04911) and by a cooperative agreement between the U.S. Environmental Protection Agency Office of Water and Michigan State University. Portions of this study were supported by a grant-in-aid for the scientific research from the Ministry of Education, Science and Culture of Japan (Grant 09306021) and the Japan Society for the Promotion of Science postdoctoral fellowship award to K.S.G. The authors thank C. Meteyer, R. Cole, and L. Creekmore for diagnostic assistance and B. Hatfield for field observations.

Supporting Information Available

A figure showing the composition of TBT and its breakdown products in sea otters (1 p) will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the Supporting Information from this paper or microfiche (105 × 148 mm, 24× reduction, negatives) may be obtained from Microforms Office, American Chemical Society, 1155 16th St. NW, Washington, DC 20036. Full bibliographic citation (journal, title of article, names of authors, inclusive pagination, volume number, and issue number) and prepayment, check or money order for $12.00 for photocopy ($14.00 foreign) or $12.00 for microfiche ($13.00 foreign), are required. Canadian residents should add 7% GST. Supporting Information is also available via the World Wide Web at URL http: //www.chemcenter.org. Users should select Electronic Publications and then Environmental Science and Technology under Electronic Editions. Detailed instructions for using this service, along with a description of the file formats, are available at this site. To download the Supporting Information, enter the journal subscription number from your mailing label. For additional information on electronic access, send electronic mail to sihelp@acs.org or phone (202) 872-6333.

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Received for review October 17, 1997. Revised manuscript received February 4, 1998. Accepted February 5, 1998.

ES970914U