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Contamination by butyltin compounds in harbour porpoise *(Phocoena phocoena)* from the Black Sea

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Abstract Concentrations of butyltin compounds (BTs) were determined in harbour porpoise *(Phocoena phocoena)* collected from the Turkish coastal waters of the Black Sea. Total butyltin compounds ($\sum$ BTs) in the liver were in the range of 89–219 ng/g on a wet weight basis. The dibutyltin (DBT) residues were higher than those of tributyltin (TBT), suggesting the degradation of TBT to DBT in the liver and the metabolic capacity comparable to other marine mammals. Any sex difference and age-dependent accumulation of BTs residues were not found in harbour porpoises, but residue levels increased until maturity and then remained constant. When compared with other marine mammals, the present results indicate that the Black Sea is also contaminated with butyltin compounds, but to a lesser degree than coastal waters of developed nations. The biomagnification factor in harbour porpoises was 0.8, which was comparable with pinnipeds and lower than cetaceans.

Dedicated to Professor Dr. Karlheinz Ballschmiter on the occasion of his 60th birthday

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Introduction

The wide application of organotin compounds such as tributlytin (TBT) in industry and agriculture has been the subject of concern over the past few decades. These chemicals have extensively been used as antifouling paints for vessels and fishing nets and also as herbicides, disinfectants, biocides for cooling systems and stabilizers for PVC plastics. With this use, considerable amounts of organotin compounds have entered the marine environment and caused toxic effects to aquatic organisms [1]. These compounds are hazardous to a wide range of aquatic organisms as evidenced by the thickening of shell and failure of spat in oysters [2], impotence of neogastropods and gastropods [3–6], reduction of dogwhelk populations [7, 8], retardation of growth in mussels [9] and immunological dysfunction in fish [10].

Despite the large amount of toxicological and residue data of organotin compounds in aquatic organisms, the monitoring studies of marine mammals have been started only recently. As the first report, butyltin compounds (BTs) were detected in various tissues of cetaceans from the western North Pacific, Bay of Bengal and Japanese coastal waters and found in significant concentrations [11, 12]. Since these findings, BTs were determined in other marine mammals, such as bottlenose dolphins from Italian coastal waters [13] and USA coastal waters [14], Risso's dolphins [15], Stellar sea lions [16] from Japan and Ganges River dolphins from India [17]. However, the BTs in higher trophic mammals from various parts of the world, especially Middle East countries, have not been sufficiently studied yet.

To understand the present status of contamination by BTs in the Black Sea aquatic ecosystem, an effort has been made in this study to determine TBT and its derivatives (DBT: dibutyltin and MBT: monobutyltin) in harbour porpoise *(Phocoena phocoena)* and its diet. To our knowledge, no information is available on the accumulation of organotin compounds in any animal from the Black Sea. Harbour porpoise is a coastal species and in

our earlier study extremely high concentrations of organochlorine residues were found in this animal [18]. This prompted us to analyse BTs in the top predator of the Black Sea ecosystem. The objectives of the present study are to elucidate the current status of contamination, possible age trend and sex differences of BTs residue levels as well as the biomagnification factor and the estimated metabolic capacity in harbour porpoise from the Turkish coastal waters of the Black Sea.

Materials and methods

Samples

The harbour porpoise and fish samples analyzed in this study were collected from Yakakent and Sinop, Turkey (Fig. 1). The porpoises were drowned in the turbot or sturgeon trammel nets during spring 1993. The exact cause of death is unknown. Liver samples of 12 female and 15 male animals of harbour porpoise were used for analysis. Available data regarding age, growth status, etc. are given in Table 1. The whole body of the diet (fish) of harbour porpoise such as whiting *(Merlangius merlangus euxinus)* and European anchovy *(Engraulis encrasicolus)* was homogenized individually for analysis. Samples were stored at –20° C until analysis. The age of harbour porpoise was determined by counting the growth layer groups in the dentine following the method of Kasuya [19].

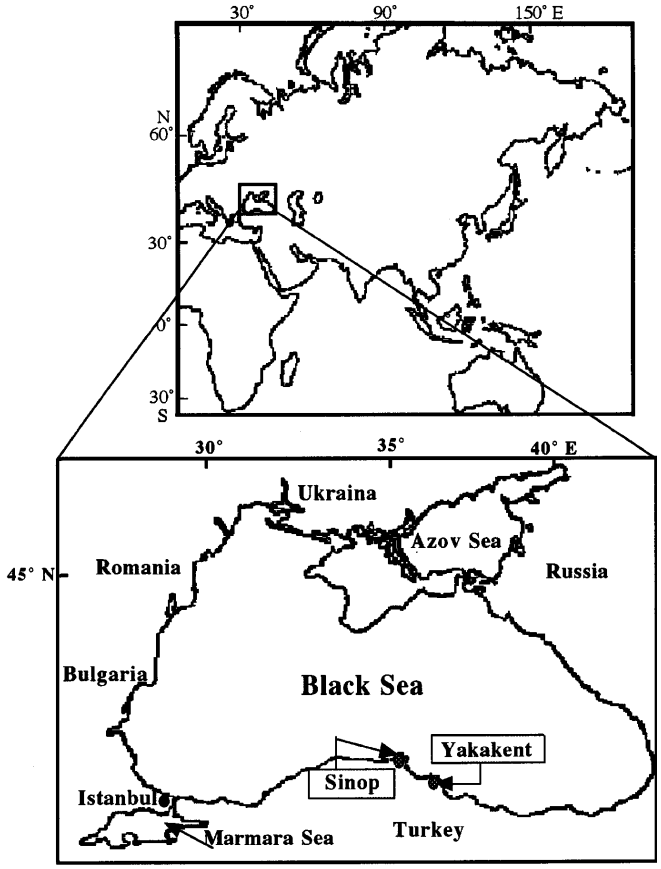

Fig. 1 Location of sampling sites

Chemical analysis

The chemical analysis for BTs was as described by Iwata et al. [12] with some modification. About 1.5 g of tissue was homogenized with 35 mL of 0.1% tropolone-acetone and 5 ml of 2 mol/L HCL. The homogenate was centrifuged at 3000 rpm and the supernatant was transferred to 0.1% tropolone-benzene. The moisture was removed with 35 g of anhydrous Na_2SO_4, concentrated to near dryness using a rotary evaporator (40° C) and made up to 5 mL with benzene. BTs in the extract were propylated by adding 5 mL of *n*-propyl magnesium bromide (2 mol/L in THF [tetrahydrofuran] solution, Tokyo Kasei Kogyo Co. Ltd., Japan) as the Grignard reagent and the mixture was shaken at 40° C for 30 min. The excess Grignard reagent was destroyed with 20 mL of 0.5 mol/L H_2SO_4 and the propylated mixture was transferred to 20 mL of 10% benzene-hexane/40 mL of hexane-washed water using 10 mL of methanol. The extract was concentrated to near dryness and made up to 5 mL with hexane. The solution was added onto a glass column (250 mm × 20 mm i.d.) packed with 20 g of dry Florisil (Wako Pure Chemical Co. Ltd, Japan) and the nitrogen was gently passed through this column for 3 h. BTs adsorbed on Florisil were eluted with 150 mL of 20% acetonitrile/water to remove fat. The eluate was collected in a separatory funnel containing 100 mL of 10% benzene-hexane/600 mL of hexane-washed water. The BTs were transferred to benzene-hexane, concentrated to 5 mL and then passed through a wet column packed with 6 g activated Florisil for final clean-up using 40 mL of hexane. The final hexane extract was concentrated to 5 mL and subjected to GC quantification.

The sample extracts were analyzed for MBT, DBT and TBT using a gas chromatograph equipped with flame photometric detector (GC-FPD). Chromatographic separation was performed on Hewlett-Packard 5890 Series II gas chromatograph with a 30 m (length) × 0.25 mm (i.d.) DB-I capillary column coated with 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° C and 270° C, respectively. The flame photometer was operated with a hydrogen-air-nitrogen flame and was equipped with a 610 nm bandpass filter selective for tin-containing compounds.

MBT, DBT and TBT of known amounts (0.2 μg/mL) were spiked into a whale liver (minke whale from the Antarctic Ocean containing undetectable levels of BTs residues), passed through the whole analytical procedure and used as an external standard. The concentrations were estimated by comparing the peak heights of butyltins in the samples with those in the external standard. Hexyl-TBT was used as an internal standard to check recovery. The reproducibility of MBT, DBT, and TBT was examined by spiking into the liver of Antarctic minke whale. Recoveries of TBT, DBT, and MBT were 108, 97, and 82%, respectively. The detection limits for MBT, DBT and TBT were 3, 1 and 1 ng/g wet weight, respectively.

Results and discussion

Current status of contamination

The butyltin compounds were detected in all the liver samples, ranging from 89 to 219 ng/g on a wet weight basis in harbour porpoise from the Black Sea (Table 1). DBT was found in the highest concentration, followed by MBT and TBT. The mean concentrations of TBT, DBT and MBT were 24, 110 and 22 ng/g on wet weight basis and mean ∑BTs concentration was 156 ng/g.

In cetaceans, higher concentrations of TBT have been mostly found in the liver, less in kidney, muscle, blubber and brain [12], which is different from organochlorines principally accumulating in lipid rich tissues such as blub-

Table 1 Concentrations of butyltin compounds (ng/g wet wt) in the liver of harbour porpoise from the Black Sea

Sample No.	Growth status	Sex	Age (GLGs)	Body length (cm)	MBT	DBT	TBT	∑BTs
1	IM	F	1	110	19	93	15	127
2	IM	F	2	105	12	114	16	142
3	IM	F	2	108	8	84	17	109
4	IM	F	2	111	19	98	20	137
5	IM	F	2	114	28	122	27	177
6	IM	F	2	120	11	83	17	111
7	IM	F	3	114	32	88	30	150
8	IM	F	3	115	35	132	42	209
9	IM	F	5	131	33	83	21	137
10	IM	F	6	120	21	121	19	161
11	MA	F	7	139	25	120	32	177
12	MA	F	10	130	27	159	19	205
13	IM	M	1	112	21	50	18	89
14	IM	M	2	110	30	81	31	142
15	IM	M	2	110	15	121	24	160
16	IM	M	2.5	111	27	127	35	189
17	IM	M	3	115	15	156	28	199
18	IM	M	3	116	21	63	21	105
19	IM	M	3	120	21	112	29	162
20	IM	M	4	114	16	94	20	130
21	IM	M	4	121	15	98	19	132
22	MA	M	4	114	27	127	35	189
23	MA	M	6	112	21	120	21	162
24	MA	M	6	123	34	152	33	219
25	MA	M	6	125	10	115	16	141
26	MA	M	7	119	15	102	20	137
27	MA	M	8	119	23	164	31	218

IM: Immature, MA: Mature, F: Female, M: Male, GLGs: Growth layer groups

Table 2 Comparison of mean concentrations (ng/g wet weight) of butyltin compounds in the liver of harbour porpoise from the Black Sea with other marine mammals

Species	Location/country	Survey year	*n*	∑BTs	References
Harbour porpoise *(Phocoena phocoena)*	Black Sea, Turkey	1993	27	156	This Study
Finless porpoise *(Neophocaena phocaenoides)*	Pacific coast of Chiba, Japan	1981	1	1120	[12]
	Seto-inland Sea, Japan	1985	1	10200	[12]
	Ise Bay, Japan	1994	1	3290	[12]
Risso's dolphin *(Grampus griseus)*	Pacific coast of Japan	1991	35	3700	[15]
Ganges River dolphin *(Platanista gangetica)*	Ganges River, India	1988–92	6	890	[17]
Bottlenose dolphin *(Tursiops truncatus)*	Mediterranean Sea, Italy	1992	2	1700	[13]
	US Atlantic/Gulf coast	1982–94	20	1400	[14]
Atlantic spotted dolphin *(Stenella frontalis)*	US Atlantic/Gulf coast	1982–94	2	350	[14]
Pygmy sperm whale *(Kogia breviceps)*	US Atlantic/Gulf coast	1991–94	3	390	[14]
Stellar sea lion *(Eumetopias jubatus)*	Alaska, USA	1976–85	38	17	[16]
	Hokkaido, Japan	1994–95	7	220	[33]

ber [20]. Hence in the present study, only liver samples were used for analysis. To understand the current status of butyltin pollution in the Black Sea, the residue levels found in the present study were compared with those of other marine mammals. As shown in Table 2, the residue levels were mostly about one order of magnitude lower in the harbour porpoise from the Black Sea than in cetaceans from US, Italian, Indian and Japanese coastal waters and were comparable to or higher than cetaceans from Hokkaido, Japan and Alaska, USA.

Table 3 Mean concentrations (ng/g wet wt) of butyltin residues in fish (diet) samples from the Black Sea

Fish (species)	*n*	MBT	DBT	TBT	∑BTs
European anchovy *(Engraulis encrasicolus)*	4	14	15	19	48
Whiting *(Merlangius merlangus euxinus)*	2	5.9	6.3	7.5	20

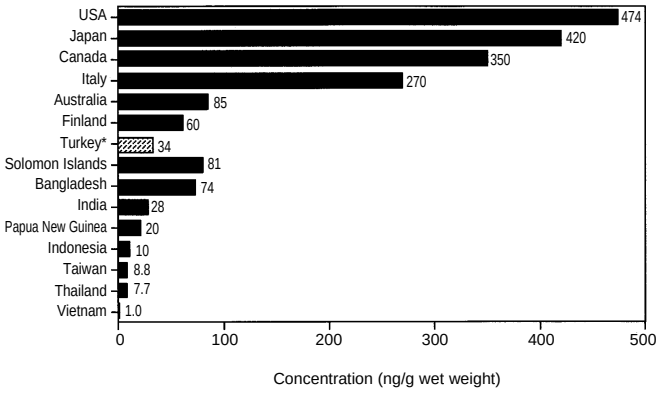

Fig. 2 Comparison of ∑BTs concentrations in fish from the Black Sea, Turkey, with those from Japan [21], USA [24], Finland [25], Asia and Oceania [22, 23] and Italy [13]. *Present study

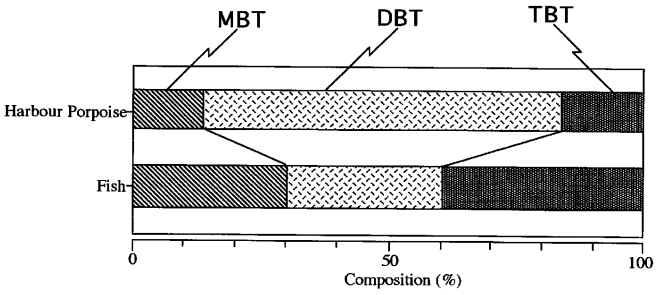

Fig. 3 Composition of butyltin compounds in harbour porpoise (liver) and fish (diet of porpoise) from the Black Sea

Besides harbour porpoise, the mean concentrations of ∑BTs in fish samples (diet) from the Black Sea was 48 ng/g wet weight for European anchovy and 20 ng/g wet weight for whiting (Table 3). When comparing the residue levels of BTs in fish from the Black Sea with those reported in literature, the present values were apparently lower than the BTs levels in developed nations and comparable to those in developing countries of Asia and Oceania (Fig. 2).

The higher concentrations of butyltin compounds in fish and marine mammals from developed nations can be explained by the extensive usage of organotins in these countries, while lower residue levels in the present animals suggest the relatively smaller usage in the surrounding countries of the Black Sea.

Composition

Dibutyltin (DBT) (71%) was the dominant component of BTs in the liver of harbour porpoise, followed by TBT (15%) and MBT (14%) (Fig. 3). A similar pattern has also been observed in pinnipeds and other cetaceans [12, 15, 16, 22] regardless of a large difference in BTs concentration among animal species. On the other hand, fish samples from the Black Sea had a TBT proportion higher than DBT and MBT (Fig. 3). It has been generally shown in many reports that TBT residue levels in fish are higher than those of DBT and MBT, irrespective of the species and sampling sites of the fish in different parts of the globe [13, 21–25].

The lesser percentage of TBT in the liver of harbour porpoise than in its diet from the Black Sea implies that a part of the TBT residues absorbed into the body of the porpoise through feeding was metabolically transformed into DBT and MBT in their liver. This means that harbour porpoise from the Black Sea has a metabolic capacity to degrade TBT in the liver, similar to other marine mammals.

Age trend and sex difference

In the case of BTs residues, no significant ($P > 0.05$, Mann-Whitney U-test) difference of BTs levels was observed between both sexes (Table 1). On the contrary, organochlorine residues in the same porpoise from the Black Sea revealed a perceptible male-female difference [18]. The concentrations of organochlorines increased with age in adult males and declined in adult females, due to their transfer to the young generation during the reproductive processes. However, BTs levels in the present study increased until maturity and then remained constant in both sexes of the porpoise (Fig. 4). A similar pattern of accumulation of butyltin residues and age trend was reported for Risso's dolphin from Japanese coastal waters [15] and bottlenose dolphins from US coastal waters [14]. Unlike the BTs residue pattern in pinnipeds [16], cetaceans including harbour porpoise from the Black Sea showed the increasing trend of butlytin in the immature animals and this is attributed to a lack of BTs excretory route such as shedding of hair and lesser degradation ca-

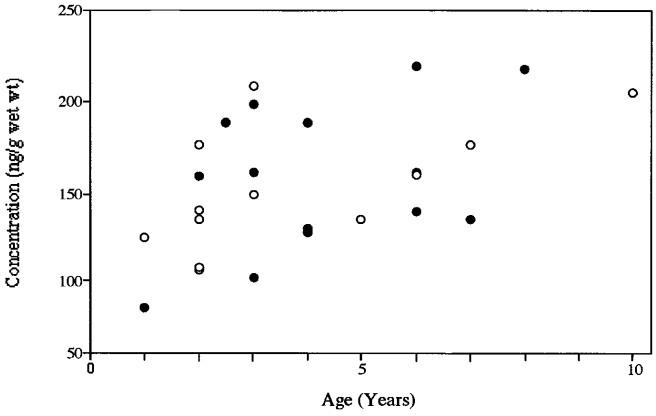

Fig. 4 Age trend of butyltins accumulation in male (●) and female (○) harbour porpoise from the Black Sea

pacity of BTs than in pinnipeds. The increasing concentrations of BTs in immature harbour porpoise from the Black Sea might be due to the larger intake of BTs rather than the metabolic degradation since younger animals feed larger for the necessity of growth. The constant feeding rate after maturity was likely to lead to steady state concentrations of BTs, which was probably balanced by the rate of metabolic BTs degradation in the body. However, no male-female difference of BTs levels suggests that these compounds are hardly transferable through gestation/location from mother to fetus/pup in harbour porpoise.

Biomagnification factor

To understand the biomagnification potential of BTs in harbour porpoise, the biomagnification factor (BMF) was calculated. The whole body concentration of BTs was estimated to calculate the BMF. The liver weight of harbour porpoise was about 3.2% of the whole body [26]. In the finless porpoise, the liver weight was 2.3% and retained 19% of the whole body burden of BTs [11]. Considering this value, the whole body residue levels were obtained by multiplying the mean residue level of BTs with the liver percentage (3.2%) of the whole body and the proportion of BTs in the liver of finless porpoise (assuming that the same burden might be occupied in harbour porpoises) as shown below:

$C_{WB} = L\% \times C_L / P_L$

C_{WB} = Whole body concentrations of ∑BTs

L% = Liver weight percentage in the body weight of harbour porpoise = 3.2%

C_L = Mean concentration of ∑BTs in the liver of harbour porpoise = 156 ng/g wet weight

$P_{L\%}$ = Proportion of liver burden of BTs in the whole body of finless porpoise = 19%

The estimated whole body BTs residue level was 26 ng/g on wet weight basis. The BMF was calculated by the concentration of BTs in the whole body of harbour porpoise and the average concentration in its diet (fish). The BMF value was 0.8, which was comparable to that of pinnipeds (BMF: 0.6) [16] and lower than that of the other cetaceans reported (BMF: 1.8–6.0) [11, 15]. Butlytin compounds are known to be metabolized by cytochrome P-450 enzymes like PB (phenobarbitol)-type in rats [27, 28]. For coastal species like harbour porpoise, the PB-type enzyme activity is higher [29, 30] when compared with other cetaceans [31, 32]. This higher PB-type enzyme activity might accelerate the degradation by BTs metabolism and lead to a lower biomagnification factor.

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