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Accumulation of Butyltin Compounds in Dolphins Stranded along the Mediterranean Coasts

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Concentrations of tributyltin (TBT) and its degradation products, monobutyltin (MBT) and dibutyltin (DBT), were determined in the liver and kidney of striped dolphins (*Stenella coeruleoalba*), bottlenose dolphins (*Tursiops truncatus*) and in a fetus of the common dolphin (*Delphinus delphi*) found stranded along the western Italian and Greek coasts in the period 1992–1994. Butyltin (BT) compounds were detected in almost all the samples analyzed and were higher in the kidney than in the liver. Total BT concentrations were 0.78–8.05 μg g^{-1} wet wt in kidney and 0.015–1.02 μg g^{-1} wet wt in liver of *S. coeruloealba*. Bottlenose dolphins had lower BT concentrations than striped dolphins. Although only one fetal sample was analyzed, it showed the highest BT concentrations of all. Unlike BT concentrations in the other adult dolphins, in the pregnant dolphin they were higher in the liver (4.35 μg g^{-1} wet wt), suggesting that BTs are transferred from mother to fetus. No significant differences in BT concentrations were found between sexes. Of the breakdown products, DBT was predominant in most liver samples and MBT was more abundant in kidney. Although BT concentrations are known to be found in cetaceans inhabiting waters of developed countries, our observations strongly suggest that concentrations found in *S. coeruleoalba* were either similar to or higher than those reported for other *Stenella* species collected from coastal areas close to harbors or marinas. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: butyltin; TBT; dolphin; Tyrrhenian sea; bioaccumulation

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INTRODUCTION

Pollution due to butyltin (BT) compounds is common in coastal and estuarine environments, and its effects on aquatic organisms have been of great concern over the last 20 years. Tributyltin (TBT) is the most toxic of these compounds, showing measurable toxic effects on marine invertebrates at concentrations as low as a few nanograms per liter (ng l^{-1}).[1] Since the mid-1960s, TBT has been widely used as a biocide in antifouling paints applied to boats and fishing nets, which are the main source of this man-made pollutant in marine coastal environments. The discovery of its toxic potential led to a ban on its use on small craft in many countries in the late 1980s.[2]

A decrease in TBT and its main breakdown products (dibutyltin, DBT and monobutyltin, MBT) has been generally observed in the environment since its ban,[3] particularly in areas dominated by the small craft (<25 m long) affected by the ban. However, in areas frequented by large ships, BT compounds continue to be detected at levels hazardous for biota.[4] Rapid bacterial degradation of TBT in water,[5] its low water solubility and relatively high affinity for particulate matter make sediments a major reservoir for this pollutant.[6] The half-life of TBT in sediments has been estimated experimentally to be 100–800 days, depending on aerobic/anaerobic conditions.[7] In deeper anoxic sediments, TBT half-life may be 1.9–3.8 years.[4] The relative persistence of TBT in sediments makes it available to benthic biota. In fact, Takahashi *et al.*[8] reported high levels of BT compounds in marine organisms collected at depths of 135–980 m in Suruga Bay (Japan).

TBT toxicity has been studied extensively in marine invertebrates. In molluscs, it produces shell thinning and sterilization of femals or so-called imposex,[9] characterizing TBT as an endocrine disrupter.[10] Embryo toxicity (i.e. arrest or delay in embryonic development) has been demonstrated in

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Figure 1 Map of Italy and Greece showing the sites of dolphin strandings.

marine bivalves[11] and benthic tunicates.[12] In mammals, the toxic effects of TBT in the form of TBT chloride (TBT-Cl) have been investigated in rats. Teratogenic effects during fetal development, related to the developmental stage at the time of exposure, were reported by Ema *et al.*[13] Exposure to TBT-Cl in early pregnancy is also reported to induce infertility in rats.[14] Snoeij *et al.*[15] observed that atrophy of the thymus and subsequent suppression of T-cell-mediated immune response in rats dosed orally with TBT-Cl was caused mainly by the metabolite DBT.

TBT has physiochemical properties such as a low water solubility (1–10 mg l^{-1}) and relatively high octanol/water partitioning coefficients (K_{ow} 5000–7000 depending on salinity) which suggest moderate bioaccumulation potential.[16] Various studies have shown that TBT accumulates in aquatic organisms, mainly those at lower trophic levels in the food web (e.g. crustaceans, molluscs, fish). Many recent studies have focused on tissue concentrations and distribution of TBT and its metabolites in marine mammals. High concentrations have been reported in dolphins,[17–20] whales,[20] porpoises[21–23] and sea lions.[24,25] These studies have shown that TBT accumulates and is biomagnified by marine mammals, irrespective of sex; accumulation is generally greater in early life and the liver appears to be the organ where the highest concentrations are found. Biomagnification factors (BMF) for TBT range from 0.6–0.8 in pinnipeds[24] to 6.0 in cetaceans.[23] This difference in the BMF has been attributed to different degradation capacities and the lack of physiological processes, such as shedding of hair in cetaceans.[17,22,25,26]

A relationship between the large-scale mortality of cetaceans and immune dysfunction caused mainly by high levels of organochlorine (OC) and BT compounds was suggested recently by Kannan *et al.*,[20] who pointed out the need for more knowledge on BT levels in dolphins affected by mass mortality. In order to understand the contamination status of BT compounds in Mediterranean cetaceans, the present study was prompted to determine the concentrations of these pollutants in the liver and kidney of dolphins found stranded along the Italian coasts. Tissues were analyzed where previous studies indicated that these pollutants accumulate preferentially in those organs. Levels were compared with those reported in other species of marine mammals from countries in which TBT is used extensively.

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Table 1 Biometry of specimens used in this study

Species	Location	Year	Sex[a]	Length (cm)	Age (years)
Stenella coeruleoalba	Northern Tyrrenian Sea	1994	M	196	7
	Northern Tyrrenian Sea	1993	M	196	9
	Northern Tyrrenian Sea	1993	M	195	—[b]
	Northern Tyrrenian Sea	1993	F	112	1
	Piombino	1993	M	173	—
	Quercianella	1993	M	200	>18
	Marina di Grosseto	1993	F	202	>18
	Northern Tyrrenian Sea	1992	F	196	18
Tursiops truncatus	Viareggio	1993	M	170	2
	Argentario	1992	—	—	—
Delphinus delphis	Kalamos (Greece)	1997	F	94	Fetus

[a] M = male, F = female.
[b] — Determination not possible.

MATERIALS AND METHODS

Samples

Liver and kidney samples of two specimens of *Tursiops truncatus* and eight specimens of *Stenella coeruleoalba* were analyzed for BT residues. The marine mammals were found standed along the Tyrrhenian coasts of Italy in the period 1992–1994 (Fig. 1). One fetus of the common dolphin (*Delphinus delphi*) found floating near its mother was also included in this study. The stillborn specimen was found in 1997 in the Ionic sea off Greece. Biometric parameters were measured and sex was determined in all specimens (Table 1). The age of the dolphins was determined by counting dental growth lines, as described by Kasuya.[27]

All samples were from fresh strandings. They were wrapped in aluminum foil, placed in solvent-washed ceramic containers, and stored at −30 °C until analysis. Care was taken to avoid contamination of samples.

Reagents and analytical procedure

Reagent grade n-hexane, benzene, acetone and hydrochloric acid and standard solutions of BTs (tributyltin chloride, dibutyltin dichloride and butyltin trichloride) were purchased from ProChem (Florence, Italy). Tropolone and Grignard reagent were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA).

The chemical analysis of BTs was carried out according to Kannan *et al.*[28] About 5 g of fresh sample was homogenized and extracted with 40 ml. of 0.1% tropolone/acetone and 10 ml of 1 M HCl. The extract was transferred to a 1000-ml separator funnel, in which 100 ml of 0.1% tropolone/benzene and 500 ml of hexane-washed water were added. After being shaken for 10 min and partitioned, the organic layer was collected and run on a column (15 mm × 350 mm) packed with 20 g sodium sulfate to remove excess water. The organic extract was then concentrated to about 2 ml in a rotary evaporator at 45 °C and the volume was made up to 5 ml with n-hexane. The extract was treated with 5 ml n-propylmagnesium bromide (Grignard reagent) to obtain the propyl derivatives of any extractable BT species present. After a 30-min reaction, the excess Grignard reagent was destroyed by addition of 20 ml 0.5 M sulfuric acid and the extract was run on a 6-g Florisil® column previously activated overnight at 130 °C. The tetra-alkyltin derivatives were obtained by elution with 40 ml n-hexane and concentrated to several milliliters for analysis by capillary gas chromatography (GC).

Chromatographic separation was carried out on an SPB-5 (5% diphenyl/95% dimethylsiloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Alkyltin species were detected by a flame photometric detector (FPD) equipped with a 610 nm bandpass filter selective for tin-containing compounds. Injector and detector temperatures were 200 °C and 270 °C respectively, while the oven temperature was programmed from 80 °C (held for 1 min) to 160 °C at a rate of 15 °Cmin^{-1} and then at a rate of 5 °Cmin^{-1} to a final temperature of 260 °C with a final hold time of 5 min. Helium at a head column pressure of 15.0 psi (103 kPa) was the carrier gas.

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Table 2 Concentrations (ng g^{-1} wet wt) of BT species in the liver and kidney of Mediterranean dolphins

Species	Tissue	*n*	MBT	DBT	TBT	ΣBTs
Stenella coeruleoalba[a]	Liver	8	97 (4.7–205)	115 (10–434)	67 (n.d.[c]–386)	259 (15–1025)
	Kidney	7	1661 (772–6596)	86 (n.d.–468)	200 (8.0–990)	2230 (783–8055)
Tursiops truncatus	Liver	2	3.0; 12	36	4.1; 15	27; 43
	Kidney	2	1003; 1968	16	5.3; 46	1024; 2014
Delphinus delphi[b]	Liver	1	5.5	333	4014	4352
	Kidney	1	2622	400	193	3215

[a] Mean and range of concentrations.
[b] Fetus.
[c] n.d. = not detected (below detection limit of 2 ng g^{-1} wet wt).

interfering compounds were tested by analysis of blanks in each batch of four samples. The recovery rates for MBT, DBT and TBT spiked into fresh samples of liver and kidney were always above 85%. The detection limit for all three BTs was below 2 ng g^{-1} wet wt for a sample of 5 g wet weight. Quantification was carried out with external standards prepared daily and BT concentrations were expressed as ng of cation per g wet wt.

RESULTS AND DISCUSSION

Concentrations of BT compounds in the liver and kidney of dolphins are reported in Table 2. The only two BT concentrations for *T. truncatus* are listed individually. BT residues were detected in almost all the samples; the highest concentrations of total BT (MBT + DBT + TBT) were found in the kidney of *S. coeruleoalba* (range 0.78–8.05 μg g^{-1} wet wt) and *T. truncatus* (1.02 and 2.01 μg g^{-1} wet wt), whereas the fetus (*D. delphis*) showed a somewhat higher BT concentration (4.35 μg g^{-1} wet wt) in the liver. This finding contrasts with previous reports,[17,21–24] in which BT concentrations have been generally higher in the liver. However, Kannan *et al.*[19] reported slightly higher BT concentrations in the kidney of Ganges river dolphins than in their liver. MBT concentrations could be the reason for this apparent discrepancy because MBT levels in liver and kidney differed by two orders of magnitude (Table 2). The greater polarity of this BT compound with respect to DBT and TBT could explain the high concentrations in the kidney. The preferential concentration of mono-substituted organotin compounds in the kidney may be due to the presence of metal-binding proteins, such as glutathione, in this organ[18] and the capacity of MBT to circulate as a cation either in the blood stream or associated with erythrocytes, as suggested by Kannan *et al.*[20]

Interestingly, BT concentrations in the fetus were high. Total BT concentrations in the liver and kidney were 4.35 and 3.21 μg g^{-1} wet wt, respectively, the highest detected in this study. To our knowledge, only one fetus of *Orcinus orca* collected in Taiji (Japan) has been analyzed for BT residues.[26] Total BT concentrations in the liver were two orders of magnitude lower than in our fetus sample.

To understand the current status of butyltin pollutions in the Mediterranean, residue levels found in our samples were compared with those of other marine mammals from elsewhere (Table 3). As shown in the table, mean BT concentrations in *S. coeruleoalba* fell within the same range as those reported for *S. frontalis* collected on the Atlantic and Gulf coasts of the USA,[20] whereas they were three to five times higher than those found in *S. longirostris* from the Bay of Bengal and the Sulu Sea,[26] two coastal areas where organotins are used on a large scale. Kannan *et al.*[20] analyzed BTs in the liver and kidney of a captive female adult (242 cm, 21 years old) *T. truncatus*, reporting values of 78 and 19 ng g^{-1} wet wt, respectively. BT concentrations in the *T. truncatus* liver in the present study were even lower than those reported by Kannan *et al.*,[20] but our concentrations in the kidney were two orders of magnitude higher. Concentrations of BT compounds measured in the kidney of bottlenose dolphins collected on the Tyrrhenian coast of Italy were at least one order of magnitude higher than those found in bottlenose

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Table 3 Mean butyltin concentrations (ng g^{-1} wet wt) in liver of marine mammals from other parts of the world

Species	Location (country)	MBT	DBT	TBT	ΣBTs	Ref.
Stenella coeruleoalba	Italian coastal waters	97	115	67	266	This study
Tursiops truncatus[a]	Italian coastal waters	3; 12	36	4.1; 15	27, 43	This study
Delphinus delphi[a,b]	Kalamos (Greece)	5.5	333	4014	4352	This study
T. truncatus	Taiji (Japan)	480	1900	470	2800	26
T. truncatus	Bay of Bengal (India)	26	44	35	110	26
S. longirostris	Bay of Bengal (India)	10	32	53	95	26
S. longirostris	Sulu Sea (Philippines)	2.0	32	21	55	26
Orcinus orca[b]	Taiji (Japan)	<4.0	14	26	40	26
Platanista gangetica	River Ganges (India)				885	19
Neophocaena phocaeoides	Japanese coastal waters	1270	2896	703	4870	23
Phoca largha		96	200	32	328	23
Mesoplodon ginkgodens		120	130	76	326	23
Phocaena phocaena	Yakakent and Sinop (Turkey)	21	110	24	156	23
Eumetopias jubatus	Hokkaido (Japan)	90	110	21	220	Kim *et al.* 1996a [17]
Grampus griseus	Pacific coast of Japan	430	2400	820	3700	Kim *et al.* 1996b [24]
T. truncatus	US Atlantic and Gulf coasts	340	960	100	1400	20
S. frontalis		94	220	44	360	20
T. truncatus	Adriatic Sea (Italy)	200	1600	400	2200	18
E. jubatus	Alaska (USA)	5.9	7.5	3.4	17	Kim *et al.* 1996c [25]
E. jubatus	Hokkaido (Japan)	75	92	17	185	25

[a] Single butyltin concentration value.
[b] Fetus.

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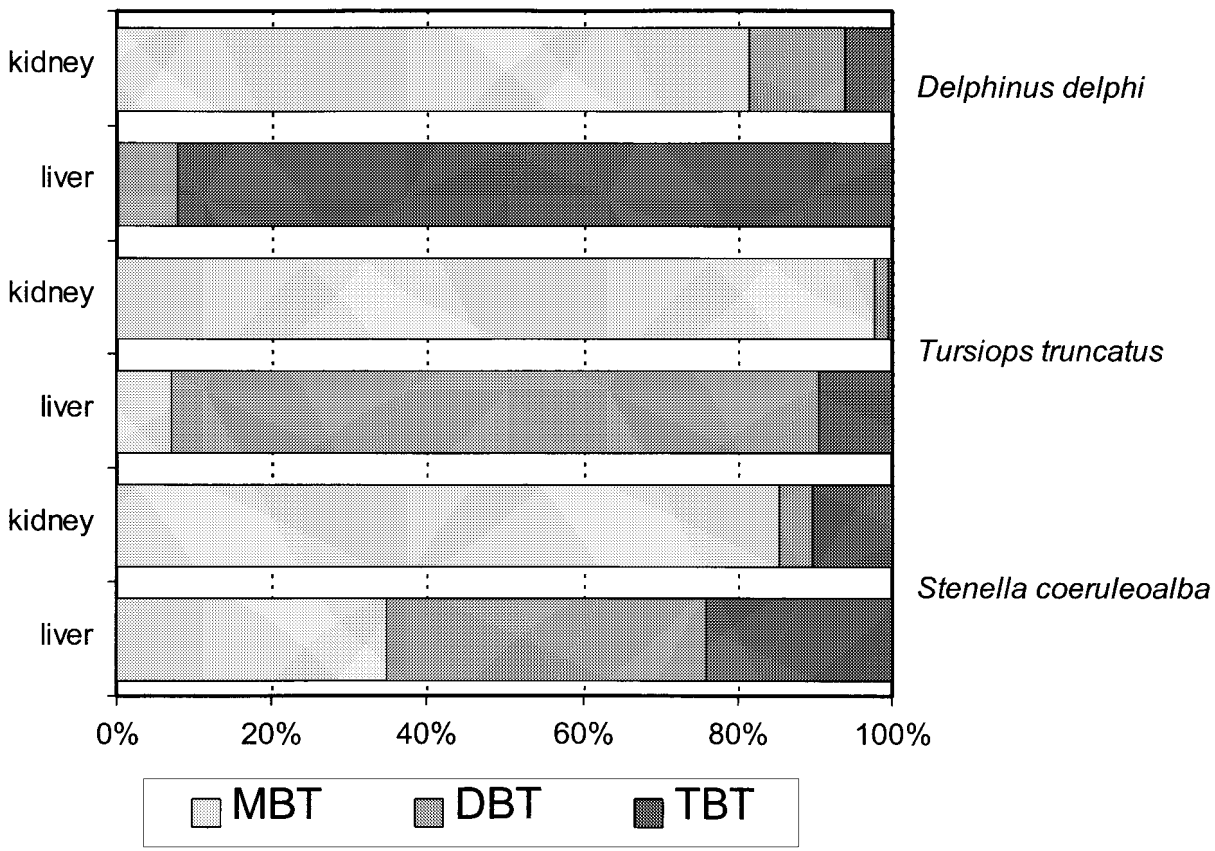

Figure 2 BT composition (relative concentrations, %) in the liver and kidney of Mediterranean dolphins.

and Atlantic spotted dolphins.[20] However, BT levels found in *T. truncatus* of that study [20] were much lower than those reported for the same dolphin species collected from the Italian coasts of the Adriatic Sea in 1992.[18] Current results show considerable contamination of dolphins, particularly *Stenella*, by this type of organic pollutant. BT concentrations recorded both in tissues of *S. coeruleoalba* and in the fetus were much higher than the values found by Kannan *et al.*[20] (Table 2).

Persistent organic contaminants such as polychlorinated biphenyls (PCBs) and DDT, regarded as immunosuppressive pollutants, have been suspected to be responsible for lowering dolphins' immunity against infectious diseases.[29,30] Kannan *et al.*[31] reported high concentrations of PCBs and DDT in stranded Mediterranean striped dolphins (*S. coeruleoalba*). At high concentrations, TBT inhibits cytochrome P450-dependent mono-oxygenases (CYP450) in fish. In vertebrates, this multienzyme system detoxifies many organic pollutants.[32,33] Its inhibition in dolphins may lead to accumulation of BT compounds and other organic pollutants (PCBs, DDT) normally detoxified by CYP450. As suggested by Kannan *et al.*,[18] high concentrations of BT compounds (1200–2200 ng g^{-1} wet wt in the liver of bottlenose dolphins), as well as of PCBs and DDT, could contribute to immune suppression in dolphins found stranded along the Atlantic and Gulf coasts of the USA. Although the current TBT concentrations found in the liver (up to 386 ng g^{-1} wet wt) and kidney (up to 990 ng g^{-1} wet wt) of striped dolphins were lower, they may pose a risk for these organisms' immune system.

The exposure of dolphins frequenting northern Tyrrhenian coastal waters to BT compounds cannot be regarded as a remote possibility because high (TBT = 260–3930 ng l^{-1}; DBT = 45–750 ng l^{-1}) and relatively constant (April–August period) levels of BTs have been found in these waters.[34] A TBT input to the marina ranging from 1.5 g

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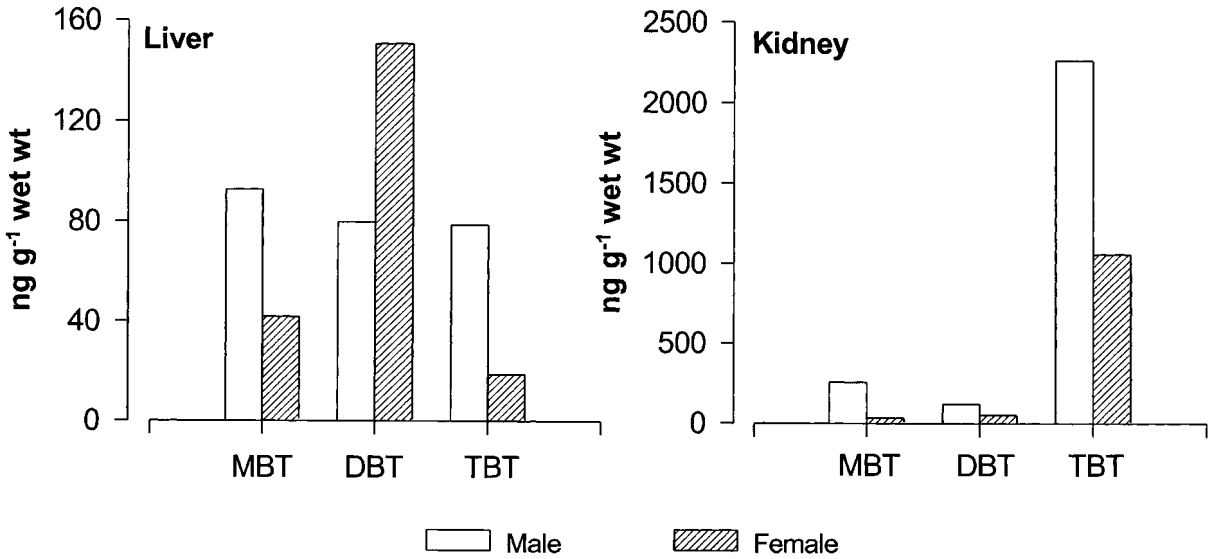

Figure 3 Mean BT concentrations in male and female specimens of *Stenella coeruleoalba*.

(pleasure boats) to 2000 g per day (large ships) was estimated. Effluents of TBT-treated cooling pipes of a thermoelectric power plant were another significant source of TBT (estimated output of TBT = 7 kg day^{-1}). Regardless of the degradation processes of TBT, the continuous input of this pollutant suggests a high and constant concentration in these waters. A more recent year-long study carried out in the Gulf of La Spezia (northern Tyrrhenian Sea) showed high DBT concentrations in water and high levels of TBT in mussel and sediment samples.[35] Significantly, this survey revealed the presence of TBT and its main breakdown products in water, sediment and mussels three years after TBT restriction in the late 1980s.[36]

BT composition (relative concentrations) in dolphin samples varied in relation to organ and species (Fig. 2). The most abundant compound was DBT in the liver samples of striped dolphins (41%) and bottlenose dolphins (83%). The prevalence of DBT in this organ has also been observed in other marine mammals, irrespective of the liver concentrations of the three chemical species.[17,18,21,23] Tanable *et al.*[26] indicated that the proportion of DBT in the liver of marine mammals from developed countries is normally 55–75% of total BTs. Besides breakdown of TBT, other sources of DBT are stabilizers for chlorinated polymers (PVC) and catalysts for some industrial processes. These common sources of DBT in developed countries could contribute to the higher concentrations of this pollutant in marine mammals. On the other hand, the high DBT concentrations in dolphin liver are not surprising, considering that CYP450 metabolizes TBT to DBT and MBT much faster than in fish.[20,37] Degradation of TBT during decomposition of the carcasses is another reason for the elevated proportion of breakdown products in the samples; our specimens had deceased a few hours before sampling.

In the fetus, the TBT concentration in the liver was higher (4015 ng g^{-1} wet wt) than that of DBT (330 ng g^{-1}) and MBT (5.5 ng g^{-1}). This pattern was also found in a fetus of *Orcinus orca*, although BT concentrations were much lower.[26] Following comparison of BT levels in the mother, the authors concluded that a transfer of BTs from the mother to the fetus was fairly unlikely. On the basis that the BT content was the same in both sexes, other authors maintain that transfer of BT from mother to fetus/infant through gestation/lactation is minimal.[20,24,25] Our results do not support this hypothesis because of the high BT concentrations, mostly TBT, found in the fetus of *D. delphis*. This single finding suggests transplacental transfer of BT compounds from the mother to the fetus, because the fetus was stillborn.

Male dolphins contained higher concentrations of BTs than females. However, the high coefficient of variation of the data (CV = 95% for liver, $n = 8$; and 91% for kidney, $n = 7$) did not enable any significant difference in the residue pattern of BT

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concentrations to be detected ($P < 0.05$, nonparametric Mann–Whitney *U*-test) between males and females of *S. coeruleoalba* (Fig. 3). Although the number of samples was too small to discern any sex-related variations, this result appears to be in line with previous studies on Risso's dolphin,[24] the Steller sea lion[17] and harbour porpoise.[23] Kannan *et al.*[20] also observed an apparently higher (but not statistically significant) BT content in male specimens of bottlenose dolphins than in females.

Although TBT is not persistent in water (half-life of the order of one to two weeks[5,38]), it is relatively persistent in anaerobic sediments so that sinks in harbour areas and estuarine sediments would persist for much longer periods. The most consistent route of exposure of dolphins to BT compounds is through the diet. Attempts have been made to estimate the biomagnification factor (BMF), or enrichment factor, in different marine mammals on the basis of their body burden of BT and that of their prey (e.g. fish, cephalopods) or on the basis of stomach contents. BMF values of 0.60, 0.80 and 6.0 have been reported for the Steller sea lion,[17] harbour porpoise[23] and Risso's dolphin,[24] respectively. The excretion rate of BT compounds in dolphins was shown to be lower than in pinnipeds.[24] This could be due to a low detoxification capacity and a lack of excretory pathways such as the shedding of fur. In fact, in a study on the tissue distribution of BTs in the Steller sea lion, the highest BT concentrations were found in fur.[25]

CONCLUSIONS

Accumulation of BT compounds was higher in Mediterranean specimens of *T. truncatus* and *S. coeruleoalba*, with respect to dolphins stranded in other coastal areas in which BTs are widely used, such as Japan. The elevated BT concentrations in these top predators, together with the low persistence of TBT in water, may indicate that these pollutants are mostly acquired directly through the diet (fish and cephalopods). Our results indicate that the presence of TBT or its metabolites in Italian coastal areas may be related to heavy shipping and the use of TBT and/or its metabolites as slimicides in the cooling pipes of thermoelectric plants. The finding of BT concentrations which were much higher in the fetus of *D. delphi* than in adult individuals suggests that transfer of BTs from the mother to the fetus is possible, perhaps as the result of a detoxification pathway of the mother. As suggested by other,[20] TBT in addition to certain organochlorine contaminants (PCBs, DDTs) may cause immune suppression in dolphins, making them more susceptible to infectious diseases, although this link is yet to be well established.

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