Environ. Sci. Technol. **1999**, *33,* 2524−2528

Distribution and Fate of Tributyltin in Surface and Deep Waters of the Northwestern Mediterranean

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Tributyltin (TBT) contamination of marine organisms collected in the open sea or in deep seawaters has been considered in several studies but not the contamination levels in these waters far from pollutant sources. This study conducted in June 1998 measured TBT concentrations in the open sea in the northwestern part of the Mediterranean. Samples were obtained in surface waters as well as along three vertical profiles between 25 and 2500 m. The analytical and sampling techniques used were calibrated to be significant for a concentration of 0.01 ng L^{-1} TBT ions. Contamination of surface waters was as high as 0.47 ng L^{-1} 20 km from shore and never lower than 0.08 ng L^{-1} in the open sea. Contamination of abyssal deep waters reached a maximum of 0.04 ng L^{-1} at a depth of 1200 m and was always significant at 2500 m. A comparison of the vertical profiles of TBT and salinity allowed assumptions to be made about the source of contamination. The presence of TBT in deep waters was attributed to the circulation of water masses during winter, while the vertical transport of particulate matter was considered to be of little importance. Contrary to results for coastal experiments, the half-life of TBT in this oligotrophic environment was estimated to be several years. The ubiquity and persistence of TBT in these waters is a new source of concern for environmentalists.

Introduction

Tributyltin (TBT) has been widely used in antifouling paints for more than 30 years. Despite the partial restrictions imposed by most countries, it is estimated that around 1200 tons of TBT yr^{-1} is used for the protection of ship hulls (*1*). High contamination of port waters has often been reported (*2*−*14*), and waters near ports have also been affected to a lesser degree (*10*). Residual contamination in the open sea has been studied less, particularly since the detection limits of available analytical methods were inadequate. In the northeastern Mediterranean, the contamination level was below the analytical threshold of 0.4 ng L^{-1} at two reference stations in the open sea (*15*). Notable concentrations have been measured in Tokyo Bay and in the Strait of Malacca where ship traffic is heavy, whereas concentrations elsewhere in the open sea have remained below the analytical threshold of 0.1 ng L^{-1} (*16*). However, indirect measurements have suggested the presence of TBT in trace amounts in oceanic waters. Analysis of squid livers and the use of bioconcentration factors have indicated that TBT contamination could reach 0.8 ng L^{-1} in waters of the Northern Hemisphere and 0.4 ng L^{-1} in those of the Southern Hemisphere (*17*). Moreover, the contamination of marine mammals constitutes an indication of TBT presence in Atlantic and Pacific waters (*18*−*20*). In the North Sea, a correlation has been found between imposex in whelks and the intensity of shipping traffic (*21*). Recently, analysis of TBT in deep-sea organisms collected from Suruga Bay, Japan, suggested that butyltin pollution has reached deep waters (*22*). In the Mediterranean, total butyltin concentrations have ranged from 1200 to 2200 ng g^{-1} in dolphin liver (*23*). All these studies tend to show the presence of trace amounts of TBT in the open sea.

The coastal waters of the northwestern Mediterranean Sea are known to be highly contaminated by TBT (*3*, *4*, *10*). The density of marinas along the Italian, French, and Spanish coasts accounts in part for this contamination, and there is also considerable commercial and naval ship traffic. No attempt has been made to measure TBT concentrations in the open sea in this area, which led us to conduct a study in surface and deep waters. An improvement in analytical detection limits allowed significant results to be obtained.

Materials and Methods

Reagents and Standards. Tri-*n*-butyltin acetate (TBT, 98%), di-*n*-butyl dichloride (DBT, 97%), *n*-butyltin trichloride (MBT, 95%), and tetraethyltin (99%) were purchased from Strem Chemical, and tetra-*n*-butyltin (TeBT, 98%) was from Fluka. Organotin stock solutions containing 100 mg L^{-1} as tin were prepared in methanol and stored in darkness at 4 °C. Working solutions were prepared daily. Methanol, *n*-pentane, and isooctane (all for pesticide analysis) were purchased from SDS−France. Nitric acid 65% Suprapur was obtained from Merck, and sodium tetraethylborate ($NaBEt_4$) was from Strem Chemical. A 4% working solution in deionized water was prepared daily. This solution was purified by three successive extractions by isooctane to obtain lower blank values.

All glassware was washed with deionized water (18 MΩ, Millipore system) before use and heated at 450 °C overnight. Reference seawater was collected far from the coasts and purified on activated carbon to remove organic contaminants.

Analyses. TBT analysis was performed by GC/FPD after ethylation in aqueous phase with $NaBEt_4$ using an adaptation of a previously described technique (*24*). Sensitivity was increased 20-fold simply by modifying the ratios of the extraction volumes and reducing the analytical blanks. The analytical protocol is shown schematically in Figure 1. Ethylation of organotin compounds was performed in a 1-L conical flask with a narrow neck (i.d. 1 cm) using a Teflon magnetic stirring rod, and the pH was adjusted to 5.5 by the addition of 65% nitric acid. An aliquot was used for electronic pH control and then discarded. $NaBEt_4$ (0.1 mL) was added, and the preparation was stirred moderately for 10 s. The reaction was allowed to develop for 10 min before extraction of the derivatives by means of a mixture of pentane (5 mL) and isooctane (0.5 mL) with magnetic stirring at 1500 rpm for 10 min. After decantation and addition of an internal standard of TeBT, the supernatant extract in the neck of the flask was concentrated under a stream of air (purified on activated charcoal) until a volume of roughly 200 μL was obtained. This extract was then analyzed by GC/FPD without preliminary purification.

A Varian 3400 gas chromatograph (GC) was used for the study. The injector was kept at 80 °C for 0.2 min, and the temperature was then increased up to 250 °C at 50 °C min^{-1}. Column temperature was maintained at 80 °C for 2 min and then increased to 220 °C at 8 °C min^{-1} . The commercial flame photometric detector was modified by adding a quartz burner and using quartz surface-induced tin emission. The

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10.1021/es981254b CCC: $18.00
Published on Web 06/24/1999

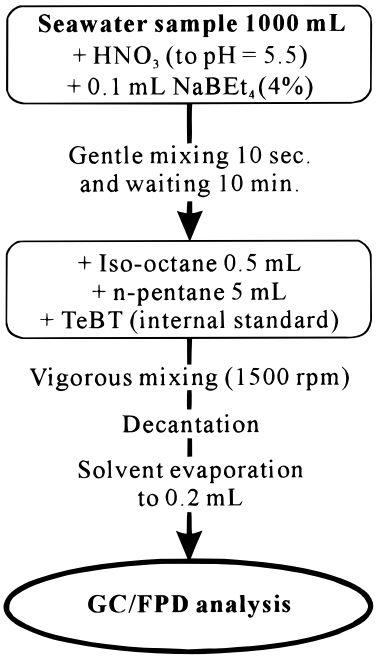

FIGURE 1. Schematic diagram of the analytical protocol.

GC was fitted with a CP-Sil 5 CB capillary column (i.d. 0.32 mm, length 25 m, film thickness 0.25 μm), the carrier gas was hydrogen (12 mL min^{-1}), and a mixture of hydrogen (45 mL min^{-1}) and air (30 mL min^{-1}) was used for the flame detector. The volume of organotin extract injected was 2 μL. Quantification was based on retention times and the peak height of external standards. All results reported in this paper are expressed as the mass of the TBT ions.

The efficiency of the method was checked. The mean blank value obtained with reference seawater was 0.035 (SD = 0.0028; $n = 6$). The theoretical detection limit calculated on the basis of 3 SD was 0.01 ng L^{-1}. The repeatability of measurements, as tested on six replicates, was ±15% for seawater spiked with 0.1 ng L^{-1} TBT ion. The quality of the blanks obtained for degradation products [monobutyltin (MBT) and dibutyltin (DBT)] was inadequate to provide significant analytical results (the corresponding data are not reported). Salinities were measured in the laboratory on a Guildline salinometer by comparing conductivity with that of normal seawater.

Sampling. Samples were obtained in June 1998 aboard the RV *Téthys* during the SUMA 2 cruise. The map of the sampling stations is shown in Figure 2. Samples were collected at a depth of 25 m for studies in surface waters. This depth is representative of Mediterranean surface waters in summer and was also chosen to avoid contamination by the ship hull and the possible influence of recent ship traffic. Sampling was performed at all stations using a polypropylene pump and a polyethylene tube (i.d. 12 mm). The sampling system was washed first with 5% HNO_3 and then carefully rinsed in situ, with elimination of the first 100 L of water pumped. The absence of contamination with this sampling system was checked by recirculation of deionized water; the TBT blank obtained was below the detection limit. Vertical profiles at stations 3, 6, and 9 were performed using 10-L all-polycarbonate Niskin bottles. Comparison of results for surface samples showed no notable differences between these two techniques. As the content of suspended particulate matter (SPM) was very low, no filtration was done to avoid any risk of contamination. The samples were stored at 4 °C in darkness in 2-L polycarbonate bottles carefully cleaned with 5% HNO_3 and then rinsed twice with ultra-clean deionized water and 3 times with samples. Analyses were performed during the month following the cruise. A second set of samples was stored in 500-mL flasks for salinity measurements. Finally, three surface water samples (A−C) were obtained in Toulon Bay and St. Tropez Bay for better determination of the gradient between the coast and the open sea.

Results and Discussion

Figure 3 shows the distribution of TBT in surface waters. TBT measurements in Toulon Bay (0.44−14.6 ng L^{-1}) and St. Tropez Bay (6.5 ng L^{-1}) were consistent with results obtained in previous cruises (*3, 4, 10*). These measurements indicate that contamination was high in the immediate vicinity of ports and docks. Stations 1−3 and 11−16, located a mean 20 km from the coast, were still subject to the influence of coastal sources of TBT contamination with concentrations between 0.13 and 0.47 ng L^{-1}. For stations 3−10 along a radial

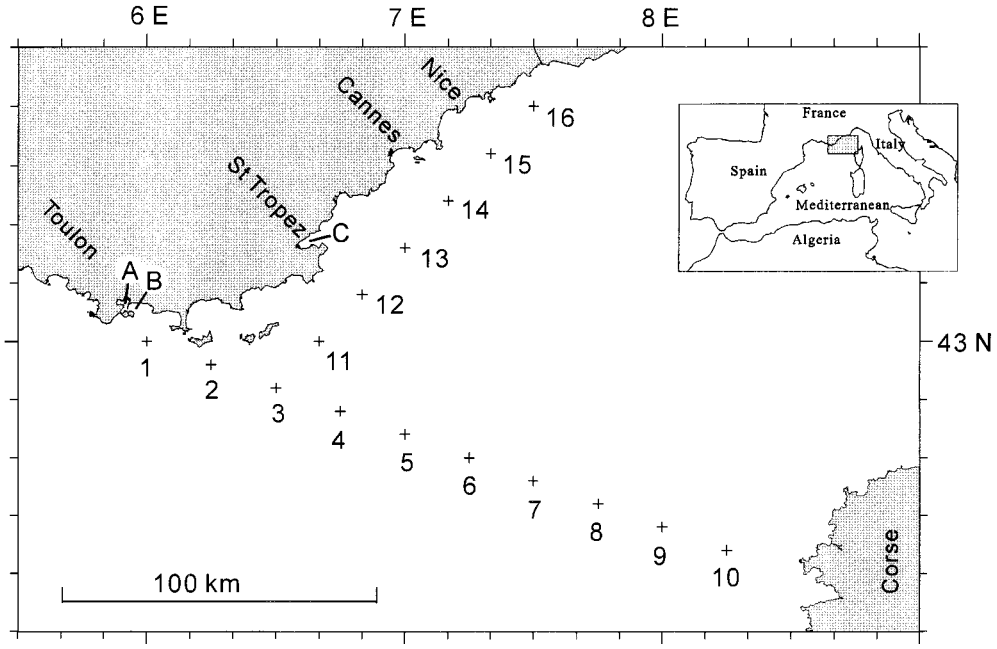

FIGURE 2. Sampling sites in the northwestern Mediterranean.

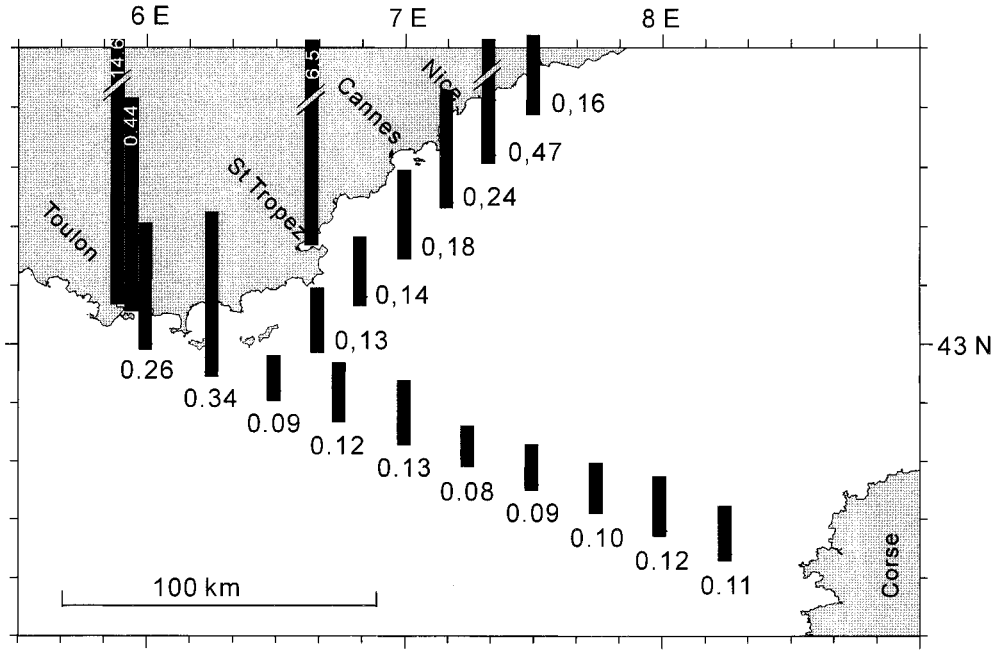

FIGURE 3. Concentrations of dissolved TBT in subsurface water (25m) samples in the northwestern Mediterranean. Units are ng L^{-1} as TBT ions.

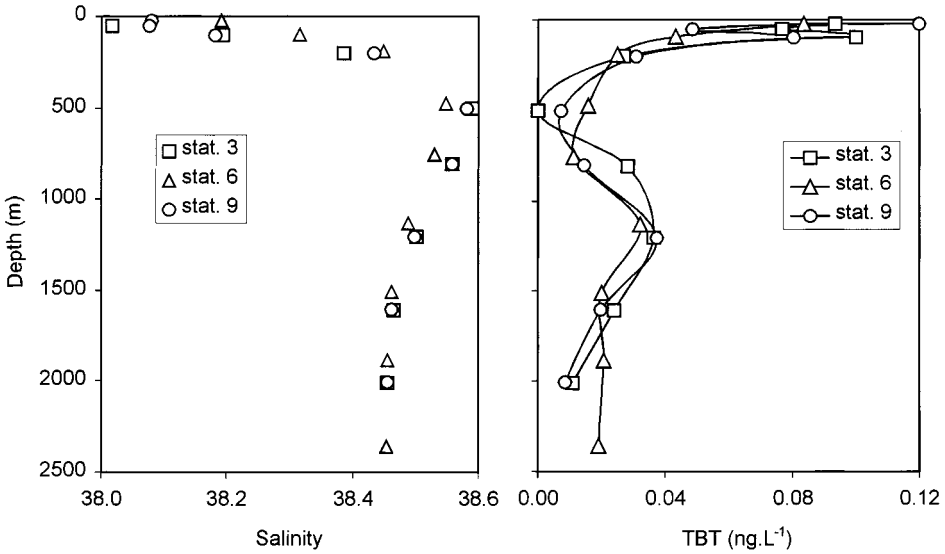

FIGURE 4. Vertical profiles for salinity (left side) and TBT (right side) at stations 3, 6, and 9. Units are ng L^{-1} as TBT ions.

between Toulon and Corsica, contamination was lower (0.08−0.13 ng L^{-1}) and more homogeneous. As these samples were obtained at a depth of 25 m, this contamination during the summer period can be considered as representative of the entire layer of surface water above the pycnocline. The profiles at stations 3 and 9 indicate that contamination was homogeneous down to a depth of 100 m in accordance with the structure of water masses in this area of the Mediterranean (*25*).

Figure 4 shows the vertical salinity and TBT profiles for deep waters at stations 3, 6, and 9. The structure of the water masses can be clearly seen with the well-defined intrusion of intermediary Levantine waters producing maximal salinity at a depth of around 500 m, as described by various authors (*25*−*29*). The lowest TBT concentrations (<0.015 ng L^{-1}) occurred at this level. These waters were formed in the eastern Mediterranean, which may be less contaminated than the region explored in the present study. Conversely, deeper waters (1200 m) supplied by descending surface waters during the winter showed a TBT contamination peak of 0.04 ng L^{-1}. This contamination was progressively reduced down to 2500 m where it was still apparent. TBT and salinity profiles for stations 3 and 9 are quite similar, whereas those for station 6 are slightly divergent.

In terms of current knowledge about marine ecotoxicology (*30*), the contamination levels measured here are low and unlikely to have direct toxic effects on marine species. However, the accumulation of organotin compounds within food chains increases the risks related to the presence of contaminant traces. Thus, high concentrations of TBT have been measured in the Mediterranean in species with a high trophic level. A maximum of 180 and 400 ng g^{-1} wet weight were found respectively in livers of a bottlenose dolphin and a blue shark, and a maximum of 170 ng g^{-1} was found in the muscle of a bluefin tuna (*23*). Recent analyses of deep-sea fish, crustaceans, cephalopods, echinoderms, and gastropods collected in Suruga Bay, Japan (*22*), showed TBT concentrations often exceeding 100 ng g^{-1} wet weight and reaching 680 ng g^{-1}. The authors of this study considered that deep-sea organisms were probably affected by contamination.

Vertical Transport. To our knowledge, TBT measurements in open seawaters and vertical profiles (including abyssal waters) have not been previously reported. This data set allows new insight into the geochemical behavior of TBT in the water column. The notion of vertical transport of TBT by particulate matter is in fact debatable. Outside of estuarial plumes, Mediterranean waters are not very turbid, with a mean SPM of 0.28 mg L^{-1} (*31*). Particulate carbon content is between 0.024 and 0.036 mg L^{-1} for waters below 200 m (*32*). The sedimentation rate on the highest abyssal plains is low (0.01 cm/yr) (*33*). The TBT partition coefficient between particles and the dissolved phase varies according to salinity, pH, and SPM content (*13*, *34*, *35*) but remains low [mean $K_D \sim 3$ (L g^{-1})]. Thus, the fraction of TBT absorbed by SPM would be less than 10^{-3}, i.e., providing a negligible theoretical capacity for vertical transport to deeper waters. The shape of the vertical profiles for dissolved TBT (Figure 4) confirms this assumption. In fact, it seems quite unlikely that TBT could have been transported vertically down to the 1200 m level without contaminating intermediary waters at the 500 m level during its passage. The presence of TBT in deep waters may be explained essentially by seasonal movements of water masses of surface origin. In winter, under the action of cold, dry winds, the surface waters are cooled, and their density becomes greater than that of the intermediary Levantine layer with which they are mixed to form the deep waters (*25*−*28*).

Degradation Kinetics. As the contamination of adjacent coastal areas of the Mediterranean has remained nearly the same over the last 6 years (*10*), the TBT measurements reported here probably reflect a steady state in the study zone, making it possible to estimate TBT degradation kinetics in the water column.

The formation of deep waters in the Liguro-Provençal Basin has been described by Béthoux (*26*). During the winter season, 27% surface waters (mean salinity 38.04) mix with 73% intermediate Levantine waters (mean salinity 38.55) to form the deep layers. This assumption of a roughly 25%/75% mixture is now generally accepted (*27*−*29*). The residence time of deep waters would thus be about 4 years, based on a thickness of surface and deep layers of around 100 and 1600 m, respectively. Given a TBT half-life of less than 30 days, this residence time would be theoretically adequate to produce concentrations below the analytical threshold. However, our observations were different. The mean TBT concentration measured in deep waters (0.025 ng L^{-1}) reflected the 25%/75% dilution of surface waters containing 0.1 ng L^{-1} TBT with very slightly contaminated intermediate waters. The hypothesis of a very slow degradation of TBT, with half-lives expressed in years rather than days, seems much more likely. This estimated half-life for TBT in open seawaters is considerably greater than that estimated to date in coastal or estuarial waters.

The degradation of dissolved TBT under the action of bacteria or light has already been considered in various experimental studies. The most commonly reported half-lives are between 4 and 19 days (*36*−*42*). This kinetics can be slowed if samples are kept in darkness (*38*), filtered or sterilized (*39*), or exposed to low temperatures (*40*), although the half-life of TBT in these particular conditions rarely exceeds 2 months. More generally, the choice a priori of experimental parameters is always a delicate affair since universally applicable simulations cannot be performed. Our study was not based on a simulation but on the observation of facts. It relates to the zone considered and is not intended for extrapolation without additional verifications. Differences with the results of previous reports (*36*−*42*) may be related to three factors: the oligotrophic nature of the study zone, the absence of light in deep waters, and the low level of contamination. Oligotrophic environments poor in organic carbon are not propitious to the development of bacteria, whose density remains very low in the entire water column and particularly in deep waters. As the estimate of bacterial density in this study was several orders of magnitude lower than that usually made for coastal waters, the kinetics of biodegradation was low. This confirms the findings of Cauwet et al. (*32*), who reported that suspended matter in this region was often less than 100 μg L^{-1} in deep waters and that particulate organic carbon (<10 μg L^{-1}) was generally very stable. The action of light was also negligible, affecting less than 1% of the water column. Finally, it is likely, with the TBT concentrations below 1 ng L^{-1} found here, that the adaptation of bacteria to the substrate could not be expressed as in previous studies in coastal areas where TBT concentrations were 100−1000 times greater.

On the basis of TBT analysis in deep-sea organisms from Suruga Bay (*22*), it was assumed that TBT degradation was slow in deep waters because of lower temperature, less penetration of sunlight, and a lack of phytoplankton. Our measurements of TBT concentrations in water allow a more quantitative approach to this problem. Our observations are original and need to be confirmed by further research. If the near-conservative and ubiquitous behavior of TBT is confirmed for other regions, this will provide additional arguments for public authorities intending to limit or forbid its use.

Acknowledgments

The authors are grateful to Dr. B. Andral and F. Galgani from IFREMER for organizing the cruise and to three anonymous reviewers for a critical reading of this manuscript.

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Received for review December 2, 1998. Revised manuscript received April 26, 1999. Accepted May 5, 1999.

ES981254B