Chemical Species of Organotin Compounds in Seawater and Their Seasonal Variations

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Tri-*n*-butyltin and its degradation products were measured in seawater samples between July 1994 and August 1995 at a marina that is located at a sequestered site of an enclosed harbor, Aburatsubo Bay, in Yokosuka, Japan. Dibutyltins, hydroxylated and oxo compounds of tributyltins and dibutyltins, carboxylated derivatives, and monobutyltin were observed in the summer months (from July to September) and only formed, if at all, in small quantities in the other seasons. Their structures were identified by comparison with synthesized authentic standards using GC/MIP/AED and four GC columns. The degradation products in the seawater in the summers between 1992 and 1995 and those in the oysters collected in the same area in the summer of 1992 showed very similar patterns. This result suggests that the degradation of tri-*n*-butyltin compounds may be attributable to microorganism flourishing in summer.

Keywords: *Tributyltin; seawater; degradation; chemical species; seasonal variation; organotin compounds*

INTRODUCTION

Organotin compounds have been produced since 1936, and the world consumption in 1989 was estimated to be 35×10^6 tons (Blunden and Evans, 1990). Among these compounds, tributyltins (TBTs) have been extensively used since the early 1970s as a biocide in antifouling paints or agents to prevent organisms from becoming encrusted on marine structures like ship bottoms, fishing nets, and docks. However, the use of these chemicals caused not only a economical loss in culturing oysters (Alzieu, 1989), but it has been determined that they may impact nontarget aquatic organisms (U'ren, 1983; Beaumont and Budd, 1984; Maguire, 1987; Lawler and Aldrich, 1987; Waldock et al., 1987; Seligman et al., 1989). Their use, therefore, has been prohibited or restricted in several countries (WHO, 1990). In addition to TBTs, monobutyltins (MBTs) and dibutyltins (DBTs) also have been used as polyvinyl chloride stabilizers and as catalysts for polyurethane foams and silicones, and in industrial processes. MBTs and DBTs, which are frequently found in seawater and freshwater environments, have been considered to derive partly from the environmental breakdown of mother compound TBTs and partly from leaching from the materials just described (Quevauviller et al., 1989; Quevauviller et al., 1991; Schebek et al., 1991).

The introduction of these chemicals into seawater has resulted in higher accumulation levels of organotin compounds in marine products. The uptake mechanism is via food chain and/or direct absorption because of the high lipophilicities (Maguire et al., 1986; Takami et al., 1987; Sasaki et al. 1988a,b; Ishizaka et al. 1989a; Suzuki et al., 1992).

Environmental fates of these organotin compounds in sludge, microorganisms, and aquatic and marine environment have been reviewed (Clark et al., 1988; Cooney, 1988; Stewart and Mora, 1990; WHO, 1990) and extensively studied thus far (Seligman et al., 1988; Francois et al., 1989; Fent et al., 1991; Fent and Hunn, 1991; Fent and Müller, 1991). In summary, the abiotic process has been considered to be less important than the biotic action, although physicochemical degradation occurs. Orsler and Holland (1982) examined the degradation of bis(tri-*n*-butyltin)oxide (TBTO), which is another form of TBT, by fungal culture filtrates. They reported that other intermediates may be formed during degradation process, in addition to dibutyltin chloride (DBTC) and monobutyltin chloride (MBTC), which are well-known degradation products. Seligman et al. (1988) also described that they obtained hydroxylated metabolites in an experiment using a labeled TBTO and a seawater, but their structures have not yet been determined.

In this work we discuss the chemical species of organotin compounds and their seasonal variations in seawater from Aburatsubo Bay, which is an enclosed yacht harbor and considered to be contaminated with butyltin and phenyltin species.

MATERIALS AND METHODS

The chemical names and their abbreviations used throughout are shown in Table 1.

Gas Chromatography/Helium Atmospheric Pressure Microwave-Induced Plasma/Atomic Emission Detection System (GC/MIP/AED). An HP model 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a split/splitless injection port interfaced to an HP model 5921A atomic emission detector equipped with a turbo makeup gas valve was used. Injections were made with an HP model 7673A automatic sampler. Four capillary columns were used: a cross-linked methyl silicone [(HP-1; Hewlett-Packard; 0.32 mm (i.d.) × 25 m × 0.17 μm (film thickness)], a cross-

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Table 1. Organotin Compounds and Their Abbreviations Used in This Paper

compound	abbreviation
n-butyltin trichloride	MBTC
di-*n*-butyltin dichloride	DBTC
n-butyl(3-hydroxybutyl)tin dichloride	D3OH
n-butyl(3-oxobutyl)tin dichloride	D3CO
tri-*n*-butyltin chloride	TBTC
n-butyl(4-hydroxybutyl)tin dichloride	D4OH
n-butyl(3-carboxypropyl)tin dichloride	DCOOH
di-*n*-butyl(3-hydroxybutyl)tin chloride	T3OH
di-*n*-butyl(3-oxobutyl)tin chloride	T3CO
diphenyltin dichloride	DPTC
di-*n*-butyl(4-hydroxybutyl)tin chloride	T4OH
di-*n*-butyl(3-carboxypropyl)tin chloride	TCOOH
triphenyltin chloride	TPTC
di-*n*-octyltin oxide	DOTO
di-*n*-octyltin dichloride	DOTC
tetrabutyltin	Bu4Sn
tri-*n*-propylethyltin	Pr3SnEt

linked 5% phenyl methyl silicone [DB-5; J&W Scientific, Folsom, CA; 0.25 mm (i.d.) × 30 m × 0.25 μm (film thickness)], a cross-linked 50% cyanopropylphenyl methyl silicon [DB-225; J&W Scientific; 0.25 mm (i.d.) × 30 m × 0.25 μm (film thickness)], and a cross-linked 14% cyanopropylphenyl methyl silicone [DB-1701; J&W Scientific; 0.25 mm (i.d.) × 30 m × 0.25 μm (film thickness)]. Operating conditions were as follows for the HP-1: column oven, programmed from 35 °C (hold 2 min) at the rate of 30 °C/min to 200 °C (hold 0 min), followed by the rate of 15 °C/min to 250 °C (hold 7 min); injection port (splitless), 250 °C; AED solvent vent off-time, 3 min. The conditions for AED were established essentially as described by Łobiński et al. (1992); namely, AED cavity temperature, 280 °C; AED cavity pressure, 1.5 psi; AED cavity scavenger gases, 3.5 kg/cm^2 (H_2) and 1.4 kg/cm^2 (O_2); AED spectrometer purge flow (N_2), 2 L/min; and wavelength for measurement, 303.319 nm. Operating conditions were as follows for the DB-5: column oven, programmed from 35 °C (hold 2 min) at the rate of 30 °C/min to 200 °C (hold 0 min), followed by the rate of 15 °C/min to 280 °C (hold 4 min); AED solvent vent off-time, 4 min; other conditions were the same with those of HP-1. All operating conditions for the DB-1701 were exactly the same as those for the DB-5. Operating conditions were as follows for the DB-225: column oven, programmed from 35 °C (hold 2 min) at the rate of 30 °C/min to 220 °C (hold 15 min); AED solvent vent off-time, 3.6 min; AED cavity temperature, 220 °C; other conditions were the same as those for HP-1.

Glassware. To avoid memory effects, all glassware was silanized with Sylon BTZ (Supelco, Inc., Bellefonte, PA) as far as possible before use.

Reagents. *n*-Hexane of pesticide grade and methylmagnesium bromide (3 M in diethyl ether) and ethylmagnesium bromide (3 M in diethyl ether) of reagent grade were purchased from Tokyo Kasei Kogyo Company, Ltd. (Tokyo). Sodium diethyldithiocarbamate (NaDDTC) of atomic absorption grade was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo) and the aqueous solution of NaDDTC (1 M) was pre-washed with *n*-hexane to remove contaminated tin compounds in the reagent and prepared daily. Ammonium chloride (NH_4Cl) of reagent grade was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo) and 500 mL of a saturated aqueous solution was pre-washed three times with *n*-hexane after addition of solid NaDDTC (1 g), and kept until use.

Authentic Standards. TBTC was purchased from Sankyo Organic Chemicals Company Ltd. (Tokyo). DBTC (>97%) and di-*n*-octyltin oxide (DOTO, of reagent grade) were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo). TPTC (98%) and tri-*n*-propyltin chloride (Pr3SnCl) were purchased from Tokyo Kasei Kogyo Company Ltd. (Tokyo) and Kanto Chemicals Company, Inc. (Tokyo), respectively. DPTC (95%), MBTC (95%), and Bu4Sn (93%) were purchased from Aldrich Chemical Company (Milwaukee, WI).

T3OH, T3CO, and T4OH were synthesized as described by Fish et al. (1976) and then purified by the method reported by Ishizaka et al. (1989b). D3OH, D3CO, and D4OH were synthesized according to the method described by Ishizaka et al. (1989b). TCOOH and DCOOH were synthesized by the method of Suzuki et al. (1992).

Sample Collection. From the summer of 1994 to the fall of 1995, the seawater samples were collected at a pier close to a marina that is situated at Aburatsubo Bay [which is long (700 m), narrow (50−100 m, especially narrow and curved in the middle), and shallow (2−5 m depth)], Yokosuka City, Kanagawa, Japan, where ~100 pleasure crafts were moored throughout all seasons. Because of its geographical features, the bay is used as a fishing boat refuge during typhoon season. At the entrance of the bay, the growth of eelgrass was observed but no plants were noted in the enclosed area. The marina is located at a sequestered site surrounded by hills (~20 m height) and therefore considered to have a slower flushing rate. The seawater increased its turbidity in July and August and its chlorophyll contents reached a maximum at 16 μg/L compared with 1 μg/L in January−February. To avoid possible contamination with microlayer waters, samples of seawater (2 L, a total of 28 samples) were collected at ~0.5 m below the surface in glass bottles at low tide at the same location throughout all seasons, at various intervals indicated in Figure 4 for 13 months. Concentrated hydrochloric acid (HCl, 36%, 10 mL) was added as a preservative. The mixtures were kept in a refrigerator (4 °C) until use within 3 days.

Solvent Extraction of Organotins from Seawater. Extraction of organotin compounds was essentially performed by following the method proposed by Dirkx et al. (1989) with a slight modification of extraction solvent (*n*-hexane in place of *n*-pentane) and equipment used. To 800 mL of seawater sample in a 1-L separatory funnel, 200 mL of a citric acid/phosphate buffer at pH 5.0 was added. If necessary, 1 N sodium hydroxide was added in a dropwise manner to adjust the pH 5.0, which was monitored by putting a drop of the sample solution on an indicator paper. Next, NaDDTC solution (1 M, 2 mL) and *n*-hexane (40 mL) were added to the seawater and the mixture was shaken for 2 min. This extraction procedure was repeated twice. The combined *n*-hexane extracts were evaporated under reduced pressure nearly to dryness at 35 °C, transferred to a Teflon-capped 50-mL centrifuge tube with the aid of diethyl ether (20 mL), and then methylated with methylmagnesium bromide (2 mL). (*Caution*: methylmagnesium bromide or ethylmagnesium bromide in diethyl ether are corrosive and a flammable combination and react violently with water.) After addition of 10 mL of water (added drop by drop until violent bubbling ceased), tri-*n*-propyl ethyltin (Pr3SnEt, 0.5 μg/mL, 50 μL), anhydrous sodium sulfite (~100 mg), saturated NH_4Cl (5 mL), and *n*-hexane (10 mL) were added, and then the mixture was shaken. After centrifugation, the organic layer was collected and the extraction procedure repeated again. The combined extracts were dried over Na_2SO_4 and then concentrated to 0.5 mL. All analyses were done in duplicate, and the data are reported as the mean of duplicate trials using two to four columns suitable for the analysis.

Standard solutions for calibration with the exception of DOTO and Pr3SnCl were prepared by methylation of organotin salts as described earlier (Suzuki et al., 1992).

DOTO (~500 mg) was weighed and dissolved in acetic acid (2 mL), and then the solution was transferred in a separatory funnel with the help of diethyl ether (100 mL). The solution was shaken vigorously with 4 N HCl (50 mL) for 15 min, and the organic phase was removed. After drying over Na_2SO_4 and evaporation of the solvent, the resulting crystalline material, dioctyltin dichloride (DOTC), was dried under reduced pressure, weighed, and dissolved in a small volume of methanol (MeOH). After dilution with *n*-hexane to 100 mL, the solution was used as a stock solution (1 mg/mL). An aliquot of stock solution was diluted with *n*-hexane and then treated as already described to give a tetrasubstituted organotin compound.

An internal standard, Pr3SnEt (100 μg/mL based on Pr3SnCl), was prepared using ethylmagnesium bromide in place of methylmagnesium bromide as just described, and the resulting stock solution of Pr3SnEt was diluted daily with *n*-hexane to afford a concentration of 0.5 μg/mL.

Recoveries (percent, mean ± SD, $n = 4$) of organotin compounds at a level of 0.5 μg/L from carbon-filtered seawater,

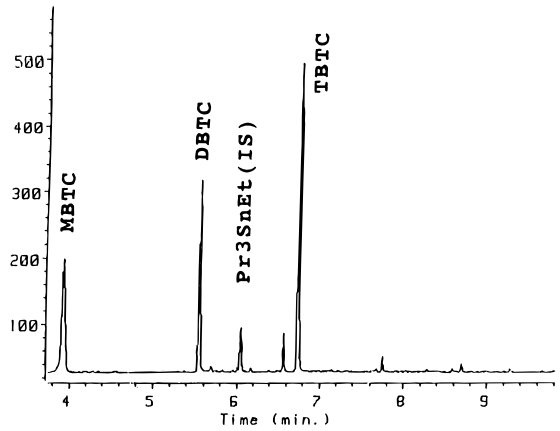

Figure 1. GC/MIP/AED chromatogram obtained from the seawater extract (August 27, 1994) on DB-225.

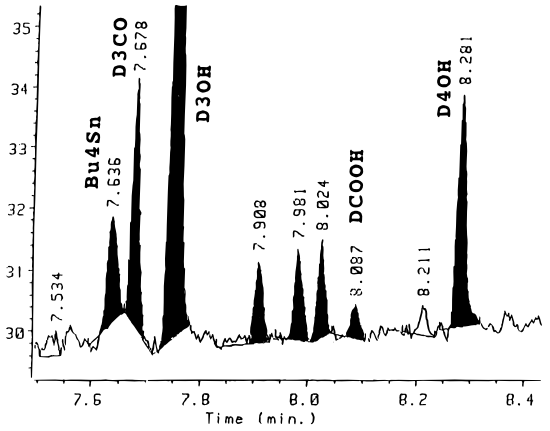

Figure 2. Enlargement of the GC/MIP/AED chromatogram (7.7−8.5 min) obtained from the seawater extract (August 27, 1994) on DB-225. Shaded peaks on gas chromatogram proved to be tin compounds on the basis of their emission spectra.

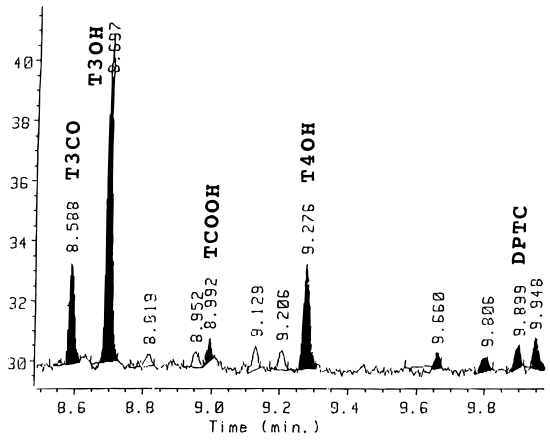

Figure 3. Enlargement of the GC/MIP/AED chromatogram (8.5−10 min) obtained from the seawater extract (August 27, 1994) on DB-225. Shaded peaks on gas chromatogram proved to be tin compounds on the basis of their emission spectra.

were 93 ± 5 (MBTC), 83 ± 2 (DBTC), 98 ± 8 (D3OH), 97 ± 5 (D3CO), 96 ± 2 (TBTC), 96 ± 7 (D4OH), 64 ± 9 (DCOOH), 97 ± 9 (T3OH), 100 ± 9 (T3CO), 88 ± 2 (Bu4Sn), 90 ± 10 (T4OH), 65 ± 5 (DPTC), 61 ± 8 (TCOOH), and 95 ± 8 (TPTC)%. Detection limits obtained on DB-5, which were calculated as three times the standard deviation of the noise levels of peak height were 0.2 (D3OH), 0.2 (D3CO), 0.2 (TBTC), 0.3 (D4OH), 0.9 (DCOOH), 0.2 (T3OH), 0.3 (T3CO), 0.2 (DPTC), 0.3 (T4OH), 0.6 (TCOOH), and 0.1 (TPTC) ng/L. With regard to MBTC and DBTC, however, the sample solutions in the blank test were contaminated with these chemicals. In spite of our exhaustive efforts to eliminate contamination, the origin could not be elucidated. A higher value was obtained in the first trial. Funnels and Erlenmeyer flasks were only lightly washed with running tap water and other small glassware was only lightly washed with acetone or running tap water followed by acetone. The glassware was reused without further modification. MBTC and DBTC levels in blank tests were 42 ± 13 ng/L (seawater basis) and 8 ± 4 ng/L (seawater basis), respectively. Several blank tests preceded analyses, and throughout a series of experiments, all glassware was washed as already described. All analytical results of MBTC and DBTC in this report were obtained by subtracting the blank values from the measured values of the seawater.

RESULTS AND DISCUSSION

For simplicity, the organotin species are referred to as the chlorides; however, this is not meant to exclude other species. All organotin concentrations throughout the experiments were expressed as chlorides (ng)/L, and analytical results were not corrected for recovery.

Identification of Organotin Compounds. A gas chromatogram on DB-225 obtained from the seawater sample collected on August 27, 1994, is shown in Figure 1. The three largest peaks [RTs (retention times): 3.928, 5.547, and 6.740 min] were easily confirmed by comparing RTs with those of authentic standards MBTC, DBTC, and TBTC, respectively. A small peak appeared at ~6.6 min, but no attempts were made to identify it. The other small peaks observed at ~7.8 and ~8.7 min are magnified in Figures 2 and 3, respectively. GC/MIP/AED spectra are usually recorded in three dimensions (time, emission wavelength, and emission intensity), and therefore relationships between time and emission intensity at a wavelength characteristic of tin (303.419 nm) were demonstrated in the chromatograms shown in Figures 1−3. On the other hand, a relationship between emission wavelength and emission intensity at the RT of an organotin compound is expressed as a emission spectrum; that is, the presence of four emission lines (300.914, 303.419, 317.505, and 326.234 nm; Łobiński et al., 1992; Suzuki et al., 1994). Depending on this principle, shaded peaks on the gas chromatogram proved to be tin compounds. The tin compounds were further characterized as Bu4Sn, D3CO, D3OH, DCOOH, and D4OH, by comparing the RTs with those of authentic specimens, as shown in Figure 2. The characterization of T3CO, T3OH, TCOOH, T4OH, and DPTC, based on RTs, is shown in Figure 3. Under these conditions, the RT of TPTC was very long and is not shown here.

The RTs and chemical assignments from the August 27, 1994 sample were studied using the DB-5, DB-225, HP-1, and DB-1701 columns, and the data are presented in Table 2. It was clearly shown that the different columns gave almost the same values, indicating that the peaks on the gas chromatograms are not overlapped with other disturbing peaks and that these assignments are correct. Therefore the analyses were, thereafter, performed with these four columns.

Monthly Variations. The monthly variations of MBTC, DBTC, and TBTC from July 1994 to August 1995 are shown in Figure 4. The TBTC level in late July, 1994 decreased until early August but rose again in late August (summer). After that, it decreased gradually and again began to increase to its highest level in late November (late fall). After it reached the lowest level in January (winter), again it began to increase and reached a maximum in June (early summer). As described by Waldock and Miller (1983) and Alzieu et al. (1989), the seasonal trends in contamination coincided with boat usage patterns and with the leaching behavior of antifouling paints; that is, the

Table 2. Comparison of Columns Used for the Determination of Organotin Compounds in Seawater Sample (August 27, 1994)[a]

column	MBTC	DBTC	D3OH	D3CO	TBTC	D4OH	DCOOH	T3OH	T3CO	Bu4Sn	T4OH	DPTC	TCOOH	DOTC	TPTC
DB-5	354	204	18.8	5.1	360	*[b]	1.7	15.2	4.3	1.8	13.6	*	*	2.6	7.3
DB-225	350	201	16.7	4.8	403	4.4	2.8	12.9	4.3	1	6.9	1.5	3.1	2.6	*
HP-1	391	178	19.6	7	*	*	0.7	15.9	4.3	2.3	7.9	2.1	3	2.4	7.8
DB-1701	407	206	19.1	5.7	318	9.5	*	*	5.1	1.9	*	1.7	*	2	6.7

[a] Results are means of duplicate measurements and expressed as chlorides (ng/L). [b] Not determined.

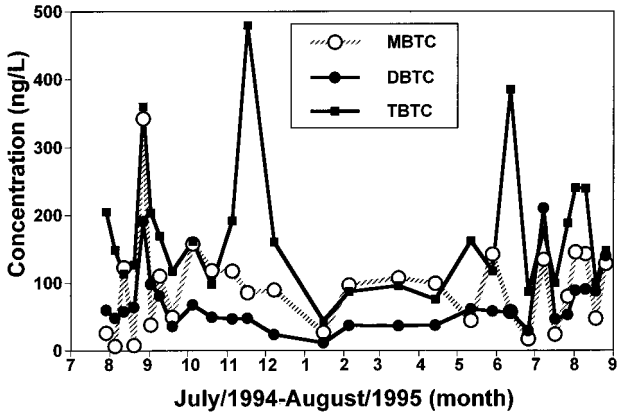

Figure 4. Seasonal variations of MBTC, DBTC, and TBTC in the samples from the Aburatsbo Bay from July 1994 to August 1995.

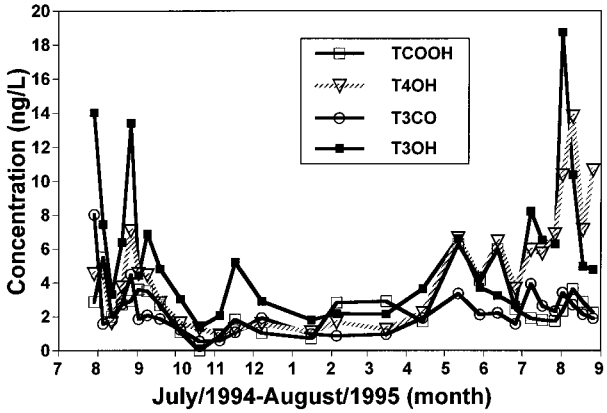

Figure 5. Seasonal variations of T3OH, T3CO, T4OH, and TCOOH in the samples from the Aburatsbo Bay from July 1994 to August 1995.

increase from the spring to the summer (May and June) would be related to the boating activity and the increase in November (fall) would be attributable to cleaning of boats in off-season to get ready for the winter. On the other hand, the increases of DBTC and MBTC were only observed in the summer (July to September), and they decreased gradually from the fall to the winter (from October to January). Furthermore, they did not exhibit any increase in mid-November (fall) when TBTC reached the highest level throughout the seasons. This result suggests that the degradation of TBTC could be associated with temperature or sunlight.

The monthly variations of T3OH, T3CO, T4OH, and TCOOH are illustrated in Figure 5. Their patterns are similar to those of MBTC and DBTC, but more closely follow the degradation pattern of TBTC. T3OH was present in the highest concentration throughout the seasons, followed by T4OH, T3CO, and then TCOOH.

The variations of dibutyltin derivatives (i.e., D3OH, D3CO, D4OH, and DCOOH) are shown in Figure 6. These compounds also exhibited patterns similar to that of DBTC. Their levels began to increase in April (spring) and show a maximum from July to late August (summer), but only a slight increase was observed in

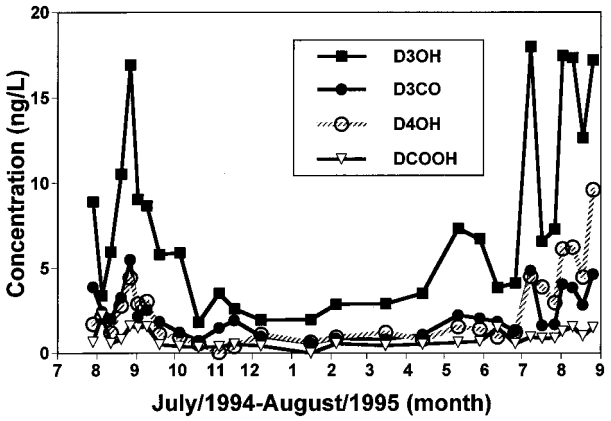

Figure 6. Seasonal variations of D3OH, D3CO, D4OH, and DCOOH in the samples from the Aburatsbo Bay from July 1994 to August 1995.

mid-November (fall) when TBTC increased dramatically, as observed for tri-*n*-butyltin derivatives just described. D3OH exhibited the highest levels of di-*n*-butyltin derivatives in July and August and the concentration was comparable to that of T3OH.

Organotin compounds that are released into the enclosed marine environment like yacht harbors are partly removed by tidal flushing and partly scavenged by particulate matter, are then fixed to the bottom mud as sediment (Waldock et al., 1987). The remained TBTC in soluble form would be quickly degraded in summer, especially in July and August. With our current knowledge, these degraded organotin compounds are very unlikely to be anthropogenic. If we assume that these oxygenated products come from industrially produced antifouling paints, they should also increase in November when TBTC concentration reached a maximum. This pattern is the opposite of our findings (Figure 6). The absence of oxygenated products at an alkyl side chain of TBTO in its formulation was also supported by the next examination. The authors could not obtain the commercially available TBTO antifouling paints (the reason is described later). When the purity of technical grade of TBTO, which is considered not to be purified after the industrial production, was examined, more than eight peaks, including MBTC, DBTC, Bu4Sn, and peaks likely due to an isomer of butyl moiety, were observed. But, there were no peaks corresponding to oxygenated products just described.

Two degradation pathways, abiotic and biotic processes, have so far been considered for TBTC in the environment. The most important degradation pathway in the biotic process has been assumed to be by microorganisms. In fact, many reports discussing the microbial degradation of TBT and the formation of hydroxylated products in cultured media have been presented. Though Barug (1981) could not isolate a pure culture that could use TBTO as a sole carbon source, he obtained cultures of bacteria and fungi that could debutylate TBTO. Barug and Vonk (1980) showed that TBTO was metabolized to CO_2 in fertile soils but not in sterilized soil samples. Olson and Brinckman

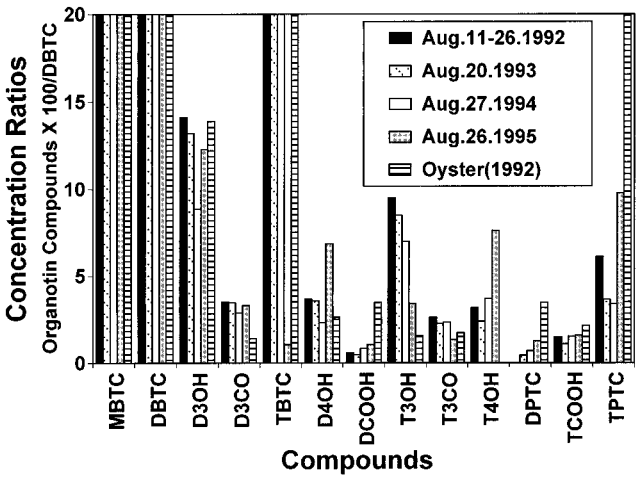

Figure 7. Concentration ratios of organotin compounds to DBTC in seawater and in oyster (organotin compounds × 100/DBTC); DBTC concentration is assumed to be 100, and the figure scale was expanded up in the longitudinal direction to a full scale of 20, so the values over 20 eventually were forced to be cut off.

(1986) showed that no biodegradation of TBT was observed in samples taken in the winter and incubated under winter conditions, but samples taken in the summer degraded TBT to DBTC and MBTC. Their results are in agreement with our experimental results that the degradation products only increased in summer and not in winter. Though not microbial, Maguire et al. (1984) reported that the green alga, *Ankistrodesmus falcatus*, debutylated TBT completely, yielding inorganic tin as well as DBTC and MBTC. Francois et al. (1989) also showed that TBTC absorbed into eelgrass was decomposed to MBTC, which was subsequently released into the surrounding seawaters. In addition to these reports, Orsler and Holland (1982), Olson and Brinckman (1986), and Seligman et al. (1988) suggested some evidence for the formation of hydroxybutyl intermediates by microorganisms, but the structures of the products were not identified. Though not a transformation in the environment, several studies have been made with respect to the degradation of TBTC or DBTC in mammals and fish. Fish et al. (1976), Kimmel et al. (1977), Ishizaka et al. (1989b), Suzuki et al. (1992), and Matsuda et al. (1993) reported that TBTs or DBTC are metabolized in vitro or in vivo by rat- or fish-liver microsome enzyme systems to MBTC, DBTC, DCOOH, and/or hydroxylated products at the 3- and 4-positions of DBTC or TBTC. These results lead us to the assumption that the degradation of TBTC in marine and fresh water environments may occur via similar pathways. The experimental results are compatible with those described, and the high degradation rates in the summers suggests that microorganisms may be involved in the breakdown of TBTC.

If these degradation products result from biodegradation by one species of microorganism or photodegradation, the ratios among degradation products concentration in each sample must always be constant. DBTC was chosen as a core substance, (i.e., a denominator) and the ratios are shown in Figure 7 (organotin compounds × 100/DBTC). Most of the seawater samples were collected at the end of August and were anticipated to show the highest levels of TBTC degradation products. As an animal example, the organotin levels in oysters have previously been reported (Suzuki et al. 1994). The ratios of D3OH, D3CO, DCOOH, T3CO, and TCOOH in the seawater samples from 1992 to 1995 showed good agreement except that the ratios of D4OH and T4OH in 1995 were approximately twice those found in 1992 to 1994. The ratios of T3OH in 1995 also deviated from the ratios of 1992−1994 by approximately one-half. These results suggests that there is a connection between DBTC concentration and degradation product concentration in each seawater sample. When degradation product concentrations are plotted versus DBTC concentrations in the seawater samples ($n = 7$) taken from July 29 to September 9, 1994, a linear correlation was obtained for each degradation product. As a result of regression analysis, the correlation coefficients were 0.8902 (MBTC), 0.8859 (D3OH), 0.7501 (D3CO), 0.8687 (D4OH), and 0.4563 (T3OH), although the correlations in other products were low at 0.2390 for DCOOH, 0.1624 for T3CO, and −0.1785 for TCOOH. The concentration of breakdown products of TBTC in seawater must be dependent on the concentration of TBTC but do not correlate with that of TBTC through seasons. The correlation between DBTC and TBTC degradation products indicates that degradation of TBTC may be influenced by other factors, most likely a microbe that flourishes in August. This season also coincided with the time of increase of chlorophyll by phytoplankton. A similar connection with TBTC degradation to chlorophyll concentrations has previously been reported (Adelman et al., 1990). Although a rough comparison, the TBTC degradation products showed similar ratios in seawater and in oysters.

Few data are available to show whether TBTC adsorbed on sediment is bioavailable and the following points suggest that it may be inaccessible: (1) anaerobic conditions surrounding the sediments, (2) higher levels of TBTC in sediment compared to seawater levels (Stallard et al., 1987), and (3) chemical forms in sediment (TBT sulfide, TBT conjugates with amino acids or protein on the nitrogen and sulfur atoms, and so on). In fact, it is well known that TBT degradation rates in sediment are slower than in the water column, particularly in anaerobic conditions. This phenomenon indicates that the degradation was not performed in sediment but in the water column, which agrees with our observation that there is no time lag between a rise in TBTC levels and an increase in the degradation products of TBTC in the summer. In light of the data just described and by analogy with the results of the animal experiments, for which there is more information, it is likely that a biodegradation pathway via hydroxylated intermediates will be realized in the environment. The proposed degradation pathways are shown in Figure 8. TBTC degrades by stepwise oxidations at the 1- or 2-position of an alkyl moiety to give DBTC and MBTC because the hydroxylated intermediates at the 1- or 2-position of an alkyl moiety of TBTC or DBTC are too unstable to be isolated and immediately give DBTC or MBTC, respectively (Fish et al., 1976). This route would be the largest degradation process in seawater. As the other degradation processes, the routes via tri-substituted oxygenated products like T3OH, T3CO, T4OH, and TCOOH due to oxidation of the 3- and 4-positions of an alkyl side chain should be mentioned. These compounds will undergo further oxidation at the 1- or 2-position of an alkyl moiety to give disubstituted oxygenated products like D3OH, D3CO, D4OH, or DCOOH together with DBTC. Disubstituted products thus formed will be degraded to MBTC or other unidentified products in a manner analogous to trisubstituted oxygenated products. As a control experiment, the kinetic study using radiolabeled TBTC in an enclosed marine ecosystem by Adelman et al. (1990) helps in understanding this pathways. These authors de-

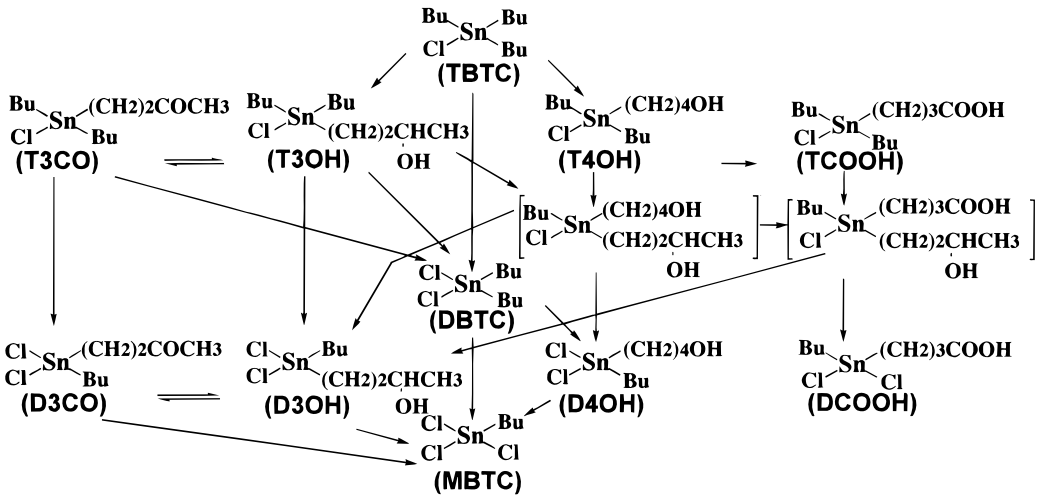

Figure 8. Proposed degradation pathways of TBTC in seawater.

scribe that most TBTC was lost from the water column through biodegradation and two-thirds of the degradation of TBTC proceeds through debutylation to DBTC, which in turn is degraded to MBTC, and one-third of the TBTC is degraded directly to MBTC. However, MBTC in the literature should include degradation products other than MBTC, except DBTC (i.e., D3OH, D3CO, etc. shown in Figure 8), depending on their assumption that MBTC is the radioactivity other than TBTC or DBTC. Their experimental results are in general agreement with the proposed pathways in Figure 8. The products formed in this way will also be rapidly fixed as sediment after being sorbed onto particulate, as in the case of TBTC.

The most important degradation pathway in the abiotic process has been assumed to be by UV irradiation. Numerous investigations have confirmed the photolytic degradation of TBTO in the environment (Maguire et al., 1983; Seligman et al., 1986; Lee et al., 1987). Because UV light only penetrates the upper few centimeters of the water column, this photodegradation process is only expected in surface waters. It has also been shown that photolysis of TBTO in environmental systems is slow, with a half-life >89 days (Maguire et al., 1983). Seligman et al. (1986) also indicated that, under natural conditions, photodegradation is less important than biological action. However, it has been generally accepted that TBTC in the marine and freshwater environment is much more concentrated in the microlayer than in water column (Cleary and Stebbing, 1987; Matthias et al., 1988). As it is expected that organotin compounds in the microlayer will be considerably more susceptible to photolytic degradation than in water column, a good deal of degradation may be anticipated in the microlayer. Although a possibility of fission on an alkyl bond (C−C, C−H) in TBTC and migration of alkyl-, OH-, and H-radicals from one molecule to another depending on photolysis could not be completely ruled out, it appears that a bond-fission between carbon and tin will precede the other fissions considering the bond energy (C−C bond, 83.6 kcal/mol; C−H bond, 98.8 kcal/mol; C−Sn bond, ∼48 kcal/mol).

TPTC had been used in antifouling paint along with TBTO since 1973 in Japan. As the use of TPTC also caused severe contamination in marine products (Ishizaka et al., 1989a), organotin-containing antifouling paints have been replaced by tin-free paints.

In the present study, TPTC reached maxima on August 20, 1994 (9 ng/L), November 17, 1994 (15 ng/L), July 17, 1995 (25 ng/L), and August 2, 1995 (23 ng/L), and a minimum from January to April, 1995 (∼2 ng/L) in seawater. The peak of TPTC coincided fairly well with those of TBTC with the exception of mid-June when TBTC showed the second highest peak. On the other hand, the peak maxima of DPTC appeared only on August 20, 1994 (3 ng/L) and July 17, 1995 (8 ng/L). Similar to the degradation products of TBTC, DPTC (a degradation product of TPTC) was only observed in summer. Further examination was not performed because of the lack of data on TPTC degradation. The coincidence of occurrence of TBTC and TPTC may be proof that they are used for the same purpose, antifouling paint. In specifications attached to a formulation of antifouling paint, which was shown to the authors by a marina worker, however, it was demonstrated to be a tin-free antifouling paint. On the other hand, our experimental results indicate TPTC and TBTC are still currently in use. This area needs additional work.

DOTC and Bu4Sn concentrations were below background levels in all samples. Their concentrations were consistently too low to allow any comment on their origin.

In conclusion, tributyltin compounds in seawater were degraded in summer to give dibutyltins, hydroxylated tributyltins, hydroxylated dibutyltins, oxo-compounds of tributyltins and dibutyltins, carboxylated derivatives, and monobutyltin. The ratios of these derivatives to dibutyltin dichloride in seawater samples between 1992 and 1995 and in oysters collected in 1992 in the same area showed similar values, indicating that microbial degradation may be an important factor in the degradation pathways of the tributyltin compounds.

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