Chemical Species of Organotin Compounds in Sediment at a Marina

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A bottom sediment collected in a marina was analyzed for organotin species, and >20 organotin compounds including biodegraded ones were confirmed by comparison with the synthesized standards using gas chromatography (GC)/mass spectrometry and a GC/atomic emission detection system. Their structures were also determined in comparison with those in a technical grade of tri-*n*-butyltin chloride (TBTC). Eleven organotin compounds were found in the technical TBTC. Among them, unexpected organotin compounds, such as di-*n*-butyl(2-ethylhexyl)tin chloride and di-*n*-butyloctyltin chloride, were identified, although the levels were low. These compounds were also found in the sediment sample. The relationship between organotin compounds in the technical TBTC and those in marine products was also discussed.

Keywords: *Organotin; TBTO; TBT; sediment; technical TBT; tributyltin; marina*

INTRODUCTION

Tributyltin compounds (TBTs) have been widely used as active ingredients in antifouling paints for ships. For this reason, adverse effects of these compounds on marine organisms, especially mollusks, were also widely reported during the 1980s. Among them, the potential of TBTs as endocrine-mimicking compounds (Smith, 1981a−d), as well the other endocrine-related toxicants, such as DDT, dieldrin, endosulfan, or toxaphene (Arnold et al., 1996a,b; Vonier et al., 1996), has become the object of public concern in recent years. TBTs have been shown to be very toxic to mollusks at nanograms per liter levels (Gibbs et al., 1987; Bryan et al., 1988).

In a previous paper (Suzuki et al., 1996), the authors reported that TBTs released into seawater are degraded to the hydroxylated and oxo compounds and those having smaller molecular weights than their mother compounds. It was also clarified that the degradation of TBTs proceeds more rapidly in summer (July, August, and September) than in the other seasons, indicating that the degradation may be due to microorganisms flourishing in summer. It is well-known that organotin compounds emitted into the enclosed marine environment are partly removed by tidal flushing and partly scavenged by particulate matter and then fixed to the bottom mud as sediment (Waldock et al., 1987). In the present study, a bottom sediment in a marina was collected and the chemical species were investigated along with those in a technical grade of tri-*n*-butyltin chloride (TBTC) for previously uncharacterized minor organotin compounds. Due to the lack of authentic standards, however, possible new organotin compounds including degradation products were synthesized. Identification was performed by gas chromatography/mass spectrometry (GC/MS) and a GC/atomic emission detection system. Furthermore, the authors also refer to the relationship between organotin compounds in the technical TBTC and those in marine products.

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MATERIALS AND METHODS

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

For the sake of brevity, each of the organotin species is referred to in the paper as if it existed only in chloride form, but this is not meant to imply the exact identities of these species in each matrix.

All organotin values in sediment are reported as the chlorides on a dry weight basis (parts per billion).

Sample Collection. A sediment sample was collected with an Ekman dredge on August 20, 1992, at a site located at the center of Aburatsubo marina in Aburatsubo Bay, Yokosuka City, Kanagawa Prefecture, Japan, and a near-surface sediment was used for analysis. The geographic features around the marina were already described in a previous paper (Suzuki et al., 1998). The sample was kept frozen in the dark at −20 °C until analysis. The sampling, sample preparation, and analytical results of the top-shell depicted in a figure in that manuscript had been already reported (Suzuki et al., 1992). The sampling, translocation from clean to contaminated areas, accumulation, extraction, and analytical results of mussels (*Mytilus graynus*) were reported in the previous paper (Suzuki et al., 1998).

Reagent. Methylmagnesium bromide [MeMgBr, 3 M in diethyl ether (Et_2O)], ethylmagnesium bromide (EtMgBr, 3 M in Et_2O), isobutylmagnesium bromide [isoBuMgBr, 1 M in tetrahydrofuran (THF)], *sec*-butylmagnesium bromide (*sec*-BuMgBr, 1 M in THF), *n*-octylmagnesium bromide (OcMgBr, 1 M in THF), *n*-butylmagnesium chloride (BuMgCl, 2 M in THF), tri-*n*-butyltin chloride (TBTC, technical grade, 95%), and 2-ethylhexyl bromide were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). Magnesium ribbon was purchased from Kishida Chemical Industries, Ltd. (Osaka). Di-*n*-octyltin oxide (DOcTO, of reagent grade) was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo).

Gas Chromatography/Helium Atmospheric Pressure Microwave-Induced Plasma/Atomic Emission Detection System (GC/MIP/AED) and GC/MS. The conditions for GC were established essentially as described by Yamamoto et al. (1997); namely, 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 make-up gas valve was used. Injections were made with an HP Model 7673A automatic sampler in the splitless mode. Four capillary

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

standard	abbrev	standard	abbrev
n-butyltin trichloride	MBTC	tri-*n*-butyltin chloride	TBTC
n-butylethyldimethyltin	BEDMeT	tri-*n*-butylmethyltin	TBMeT
n-butylisobutyltin dichloride	BisoBTC	*n*-butyl(4-hydroxybutyl)tin dichloride	D4OH
n-butylisobutyldimethyltin	BisoBDMeT	*n*-butyl(2-ethylhexyl)tin dichloride	BEHTC
n-butyl-*sec*-butyltin dichloride	B*sec*BTC	*n*-butyl(3-carboxypropyl)tin dichloride	DCOOH
n-butyl-*sec*-butyldimethyltin	B*sec*BDMeT	*n*-butyl(2-ethylhexyl)dimethyltin	BEHDMeT
di-*n*-butyltin dichloride	DBTC	*n*-butyl-*n*-octyltin dichloride	BOcTC
di-*n*-butyldimethyltin	DBDMeT	*n*-butyldimethyloctyltin	BDMeOcT
di-*n*-butymethyltin chloride	DBMeTC	di-*n*-butyl(3-hydroxybutyl)tin chloride	T3OH
di-*n*-butylethylmethyltin	DBEMeT	di-*n*-butyl(3-oxobutyl)tin chloride	T3CO
2-ethylhexyltin trichloride	MEHTC	tetrabutyltin	Bu4Sn
2-ethylhexyltrimethyltin	MEHMeT	diphenyltin dichloride	DPTC
tri-*n*-propyltin chloride	TPrTC	di-*n*-butyl(4-hydroxybutyl)tin chloride	T4OH
ethyltri-*n*-propyltin	ETPrT	di-*n*-butyl(3-carboxypropyl)tin chloride	TCOOH
n-octyltin trichloride	MOcTC	di-*n*-butyl(2-ethylhexyl)tin chloride	DBEHTC
trimethyl-*n*-octyltin	TMeOcT	di-*n*-butyl(2-ethylhexyl)methyltin	DBEHMeT
n-butyl(3-hydroxybutyl)tin dichloride	D3OH	di-*n*-butyl-*n*-octyltin chloride	DBOcTC
n-butyl(3-oxobutyl)tin dichloride	D3CO	di-*n*-butylmethyl-*n*-octyltin	DBMeOcT
di-*n*-butylisobutyltin chloride	DBisoBTC	di-*n*-octyltin oxide	DOcTO
di-*n*-butylisobutylmethyltin	DBisoBMeT	di-*n*-octyltin dichloride	DOcTC
di-*n*-butyl-*sec*-butyltin chloride	DB*sec*BTC	triphenyltin chloride	TPTC
di-*n*-butyl-*sec*-butylmethyltin	DB*sec*BMeT	trimethyltin chloride	TMeTC
bis(tri-*n*-butyltin)oxide	TBTO	*n*-butyldimethyltin chloride	BDMeTC

Table 2. Optimal GC and AED Temperature Parameters

	GC/AED columns				GC/MS column
	DB-5[a]	DB-225[b]	DB-1701[c]	HP-1[d]	HP-1[e]
GC Parameters					
injection port temp (°C)	250	220	250	250	250
head pressure (kPa)	173	173	173	142	20
solvent vent-off time (min)	4.0	3.6	4.0	3.0	
oven program					
initial temp (°C/hold time, min)	35/2	35/2	35/2	35/2	35/2
ramp rate 1 (°C/min)	30	30	30	30	30
middle temp (°C/hold time, min)	200/0		200/0	200/0	200/0
ramp rate 2 (°C/min)	15		15	15	15
final temp (°C/hold time, min)	280/4	220/15	280/4	280/6	250
Interface and AED Temperature Parameters					
transfer line (°C)	280	220	280	280	
cavity (°C)	280	220	280	280	

[a] A cross-linked 5% phenyl methyl silicone [J&W Scientific, Folsom, CA; 0.25 mm (i.d.) × 30 m × 0.25 μm (film thickness)]. [b] A cross-linked 50% cyanopropylphenyl methyl silicone [DB-225; J&W Scientific; 0.25 mm (i.d.) × 30 m × 0.25 μm (film thickness)]. [c] A cross-linked 14% cyanopropylphenyl methyl silicone [J&W Scientific; 0.25 mm (i.d.) × 30 m × 0.25 μm (film thickness)]. [d] A cross-linked methyl silicone [Hewlett-Packard; 0.32 mm (i.d.) × 25 m × 0.17 μm (film thickness)]. [e] A cross-linked methylsilicone [Hewlett-Packard; 0.2 mm (i.d.) × 12 m × 0.3 μm (film thickness)].

columns were used, and the operating conditions are shown in Table 2. The conditions for AED, except for temperature and solvent vent-off time, were established essentially as described by Łobiński et al. (1992); namely, 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 wavelengths for measurement at 303.319 nm.

GC/MS spectra were obtained by an HP 5917A (Hewlett-Packard Co.) in the electron-impact mode at an ionization voltage of 70 eV. The column used and operating temperatures are listed in Table 2. Other conditions were as follows: radiator temperature (MS temperature), 185 °C; mass filter, 150 °C. Mass spectrum was measured in principle at a maximum of each peak.

Qualitative measurement of each compound was done by selective ion monitoring (SIM) using the indicated fragment ions with 100 ms of dwell time for each ion. Scanning for each ion was performed from a high mass field to a low mass field.

For simplicity, molecular weights (*M*) of organotin compounds on GC/MS are expressed on the basis of a tin atomic weight of 120, which constitutes a maximum amount (32.97%) in 10 stable isotopes of tin.

Authentic Standards. *TBTC* (of analytical grade), *di-n-butyltin dichloride* (DBTC, of analytical grade), and *tri-n-propyltin chloride* (TPrTC, of analytical grade) were purchased from Kanto Chemicals Co., Inc. (Tokyo). *Triphenyltin chloride* (TPTC, 98%) was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo). *Diphenyltin dichloride* (DPTC, 95%), *n-butyltin trichloride* (MBTC, 95%), *tetrabutyltin* (Bu4Sn, 93%), *dimethyltin dichloride* (DMeTC, 97%), and *trimethyltin chloride* (TMeTC) were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Di-n-butyl(3-hydroxybutyl)tin chloride (T3OH), *di-n-butyl-(3-oxobutyl)tin chloride* (T3CO), and *di-n-butyl(4-hydroxybutyl)tin chloride* (T4OH) were synthesized as described by Fish et al. (1976) and then purified according to the method reported by Ishizaka et al. (1989a). *n-Butyl(3-hydroxybutyl)-tin dichloride* (D3OH), *n-butyl(3-oxobutyl)tin dichloride* (D3CO), and *n-butyl(4-hydroxybutyl)tin dichloride* (D4OH) were synthesized according to the method described by Ishizaka et al. (1989a). *Di-n-butyl(3-carboxypropyl)tin chloride* (TCOOH) and *n-butyl(3-carboxypropyl)tin dichloride* (DCOOH) were synthesized according to the method of Suzuki et al. (1992). *Dioctyltin chloride* (DOcTC) was prepared from DOcTO according to the method of Suzuki et al. (1996).

Previously uncharacterized organotin compounds were synthesized, and the outline of synthetic routes of them is illustrated in Figure 1.

Di-n-butylmethyltin Chloride (DBMeTC) and n-Butyldimethyltin Chloride (BDMeTC) (Mixture A). The synthesis of alkyltin chlorides having different alkyl moieties on the tin

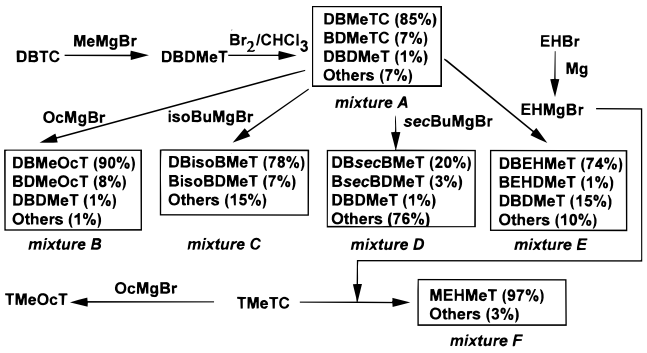

Figure 1. Synthetic routes of organotin compounds.

atom was principally performed as described elsewhere (Ishizaka et al., 1989a). Namely, di-*n*-butyldimethyltin (DBDMeT, 1.0 g), which was obtained from di-*n*-butyltin dichloride (DBTC) and MeMgBr, was treated with Br_2 in chloroform to afford a crude oil (1.3 g) after evaporation of solvent. After repeated purification on Florisil (10 g; Floridin Co., Hancock, WV) using Et_2O followed by 1% acetic acid (AcOH) in Et_2O, 1% AcOH in Et_2O fractions were combined and evaporated to give mixture A (0.8 g). The composition of mixture A was determined as follows. Alkylation was conducted as will be described under Extraction of Organotins from Sediment. The treatment of mixture A (20 μg) with BuMgCl gave a mixture of tri-*n*-butylmethyltin (TBMeT, 85%), DBDMeT (8%), and others (7%), which was calculated on the basis of the sum of all tin peak areas on HP-1 using GC/MIP/AED. On the other hand, the treatment of mixture A (20 μg) with EtMgBr afforded a mixture of di-*n*-butylethylmethyltin (DBEMeT, 85%), *n*-butylethyldimethyltin (BEDMeT, 7%), di-*n*-butyldimethyltin (DBDMeT, 0.6%), and others (7.4%). Depending on these results, the approximate composition of mixture A was determined to be DBMeTC (85%), BDMeTC (7%), DBDMeT (1%), and others (7%).

Di-n-butylmethyloctyltin (DBMeOcT) and n-Butyldimethyloctyltin (BDMeOcT). Mixture A (20 μg) was alkylated as usual using OcMgBr as an alkylating reagent to afford a mixture of DBMeOcT, BDMeOcT, DBDMeT, and others (mixture B) as shown in Figure 1.

Di-n-butylisobutylmethyltin (DBisoBMeT) and n-Butylisobutyldimethyltin (BisoBDMeT). Mixture A (20 μg) was treated with isoBuMgBr to give a mixture of DBisoBMeT, BisoBDMeT, and others (mixture C).

Di-n-butyl-sec-butylmethyltin (DBsecBMeT) and n-Butyl-sec-butyldimethyltin (BsecBDMeT). The reaction of mixture A (20 μg) with *sec*BuMgBr afforded a mixture of DB*sec*BMeT, B*sec*BDMeT, and others (mixture D).

Trimethyl-n-octyltin (TMeOcT). The reaction of TMeTC with OcMgBr yielded TMeOcT.

Di-n-butyl(2-ethylhexyl)methyltin (DBEHMeT) and n-Butyl-(2-ethylhexyl)dimethyltin (BEHDMeT). Mixture A was reacted at 50 °C with 2-ethylhexylmagnesium bromide (EHMgBr, 2 mL), which was made from 2-ethylhexyl bromide (15 g, 0.08 mol) and magnesium ribbon (2 g, 0.11 mol). The obtained extract consists of a mixture of DBEHMeT, BEHDMeT, DBDMeT, and other products (mixture E).

2-Ethylhexyltrimethyltin (MEHMeT). TMeTC was treated with EHMgBr as usual to give a mixture of MEHMeT and others (mixture F).

All organotin compounds used were are listed in Table 1, and mass spectral data of synthesized organotin compounds are shown in Table 3.

Preparation of Matrix Solution. To equalize, as far as possible, the matrix effects of sample solutions with those of standard solutions in GC/MIP/AED measurement, a matrix solution was added to both sample solutions and standard solutions, respectively. The matrix solution was prepared according to the method of Yamamoto et al. (1997). A 3% sodium chloride solution (400 mL) was added to 200 g of rainbow trout muscle, which is available throughout the year, and the mixture was homogenized and then extracted with *n*-hexane (300 mL). After centrifugation, the water layer was again extracted with Et_2O (300 mL) and centrifuged. The combined mixture of organic layer was filtered through a filter paper if necessary and then concentrated to 40 mL.

Extraction of Organotins from Sediment. After the addition of AcOH (10 mL) to sample (2 g of sediment on wet weight basis) in a centrifuge tube (50 mL), the mixture was allowed to stand overnight. After the addition of water (10 mL) and 0.5% tropolone benzene (15 mL), the centrifuge tube was tightly capped and then vigorously shaken for 30 min (*caution: benzene is a carcinogen*). After centrifugation at 2050*g* for 15 min, the upper layer was removed and another portion of benzene (5 mL) was slowly run down the wall of the centrifuge tube. After removal of the benzene layer, the combined organic layer was concentrated nearly to dryness under reduced pressure. To remove acetic acid in the residue, *n*-hexane (20 mL) was added and again evaporated nearly to dryness under reduced pressure. The residue was dissolved in a small amount of *n*-hexane/EtOAc mixture (2:1 v/v) and transferred to a Pasteur pipet minicolumn containing 1 g of HCl-treated silica gel (Wakogel C-100) (Hattori et al., 1984) prepared with *n*-hexane. After elution with *n*-hexane/EtOAc (2:1 v/v; 20 mL), the eluate was evaporated under reduced pressure and EtOAc remaining was removed by the addition of *n*-hexane (20 mL) and followed by further evaporation. The residue was taken up in Et_2O (20 mL) and subjected to methylation with MeMgBr (4 mL). After conventional treatment, the reaction mixture was extracted twice with *n*-hexane (10 mL) and the combined extracts were concentrated under reduced pressure below 35 °C to exactly 1 mL and kept at −20 °C until analysis after addition of crystalline Na_2SO_3 (∼100 mg) (Sasaki et al., 1988).

Standard solutions for calibration, with the exception of ethyltri-*n*-propyl tin (ETPrT), were prepared by methylation of organotin salts as described earlier (Suzuki et al., 1992). Briefly, diluted solutions of TCOOH and DCOOH in acetone (20 μg/2 mL, each), diluted standard solutions of MBTC and DBTC in *n*-hexane (5 μg/0.5 mL, each), TBTC in *n*-hexane (10 μg/1 mL), and others in *n*-hexane (20 μg/2 mL, each) were put into a flask (100 mL) containing 4 drops of 4 N HCl. The solvent was distilled off nearly to dryness under reduced pressure below 35 °C, and then the residue was transferred to a 50-mL centrifuge tube with Et_2O and made up to ∼20 mL by the addition of Et_2O. After methylation, the reaction mixture was treated with H_2O (10 mL), saturated NH_4Cl (6 mL), and crystalline Na_2SO_3 (∼200 mg) and then extracted with *n*-hexane (6 mL × 2). The extract was dried over Na_2SO_4 and then concentrated to exactly 10 mL. This solution was used as a stock solution after the addition of crystalline Na_2SO_3 (∼200 mg) as a stabilizer. An internal standard, ETPrT (20 μg/mL as TPrTC) was prepared by using 200 μL of TPrTC (1000 μg/mL) and 2 mL of EtMgBr in place of MeMgBr. To each 400, 200, 100, and 50 μL of the stock solution was added an internal standard (ETPrT; 0.5 μg/mL, 200 μL), matrix solution (200 μL), and *n*-hexane to make the volume up to 2 mL, and these solutions were used as mixed working standards. Standards and sample solutions thus prepared were kept at −20 °C.

To an aliquot of the sample solution in a small test tube was added ETPrT (0.5 μg/mL, 50 μL), matrix solution (50 μL), and *n*-hexane to make the volume up to exactly 500 μL, and then the mixtures were sent to autosampler vials of 100-μL capacity for GC/MIP/AED analysis. The organotin concentrations reported here represent the mean values of triplicate determinations and are not corrected for reagent blank.

Recoveries (percent, mean ± SD, $n = 3$) of organotin compounds spiked to Kanto loam soil at a level of 0.1 μg/g of sample were 37.0 ± 13.0 (MBTC), 112 ± 5.5 (DBTC), 99.6 ± 7.6 (D3OH), 105 ± 5.9 (D3CO), 116 ± 6 (TBTC), 91.0 ± 4.7 (D4OH), 82.0 ± 11.0 (DCOOH), 117 ± 3 (T3OH), 111 ± 5 (T3CO), 69.0 ± 9.2 (Bu4Sn), 119 ± 8.5 (T4OH), 118 ± 30 (TCOOH), 124 ± 5 (DPTC), 115 ± 11 (DOcTC), and 92.4 ± 16 (TPTC), respectively.

Table 3. Mass Spectral Data of Synthesized Organotin Compounds (Base Peak: 100)

compound	*m/z* (relative intensity)
DBMeOcT	305 (M − 15, OcBuMeSn$^+$, 12), 263 (M − 57, OcMe$_2$Sn$^+$, 100), 249 (OcMeHSn$^+$, 10), 207 (BuMe$_2$Sn$^+$, 36), 193 (BuMeHSn$^+$, 16), 177 (BuSn$^+$, 2), 151 (Me$_2$HSn$^+$, 96), 137 (MeH$_2$Sn$^+$, 11), 135 (MeSn$^+$, 26), 121 (HSn$^+$, 9)
DBisoBMeT	292 (M − 15, 1), 249 (M − 57, 54), 193 (BuMeHSn$^+$ and isoBuMeHSn$^+$, 100), 177 (BuSn$^+$ and isoBuSn$^+$, 12), 137 (MeH$_2$Sn$^+$, 44), 135 (MeSn$^+$, 53)
BisoBDMeT	249 (M − 15, 8), 207 (M − 57, 66), 193 (BuMeHSn$^+$ and isoBuMeHSn$^+$, 24), 151 (Me$_2$HSn$^+$, 100), 137 (MeH$_2$Sn$^+$, 25), 135 (MeSn$^+$, 33), 121 (HSn$^+$, 15)
DB*sec*BMeT	249 (M − 57, 51), 193 (BuMeHSn$^+$ and *sec*BuMeHSn$^+$, 100), 137 (MeH$_2$Sn$^+$, 38), 135 (MeSn$^+$, 58), 121 (HSn$^+$, 22)
B*sec*BDMeT	207 (M − 57, 22), 151 (Me$_2$HSn$^+$, 100), 135 (MeSn$^+$, 19), 121 (HSn$^+$, 9)
TMeOcT	263 (M − 15, 44), 165 (Me$_3$Sn$^+$, 100), 151 (Me$_2$HSn$^+$, 76), 135 (MeSn$^+$, 24), 121 (HSn$^+$, 8)
BEHDMeT	263 (M − 57, EHMe$_2$Sn$^+$)
MEHMeT	263 (M − 15, 42), 165 (Me$_3$Sn$^+$, 100), 151 (Me$_2$HSn$^+$, 98), 135 (MeSn$^+$, 39), 121 (HSn$^+$, 12)

Detection limits in the soils were 0.5 ng/g for MBTC (DB-5), 0.1 ng/g for DBTC (DB-5), 0.4 ng/g for D3OH (DB-5), 0.6 ng/g for D3CO (DB-5), 0.4 ng/g for TBTC (DB-5), 0.6 ng/g for D4OH (DB-1701), 1.5 ng/g for DCOOH (DB-5), 0.8 ng/g for T3OH (DB-5), 0.6 ng/g for T3CO (DB-5), 0.6 ng/g for T4OH (DB-5), 0.5 ng/g for Bu4Sn, 1.6 ng/g for TCOOH (HP-1), 1.2 ng/g for DPTC (HP-1), 1.0 ng/g for DOcTC, and 1.2 ng/g for TPTC (DB-5).

RESULTS AND DISCUSSION

Syntheses of Organotin Compounds. Organotin compounds used for identification were in part synthesized by removing an alkyl group with Br_2 in $CHCl_3$ from tetraalkylated organotin compounds. Consequently, they gave a mixture of trialkylated organotin compounds. A series of experiments has shown that in organotin compounds of the type $(R_a)_2(R_b)_2Sn$ ($R_a > R_b$) the separation of produced trialkylated organotin chlorides into each component is extremely difficult and that a smaller alkyl moiety (R_b) is preferentially removed. When DBDMeT was used as a starting material, a mixture of DBMeTC (85%), BDMeTC (7%), DBDMeT (1%), and others (8%) (mixture A) was obtained. Their structures and relative ratios were determined by tetraalkylation of mixture A with BuMgCl and EtMgBr. The structures of tetraalkylated organotin compounds produced by the reaction of mixture A with the other alkylating reagents were confirmed by GC/MS and RTs on GC/MIP/AED because GC/MS patterns of simply alkylated tetraalkyltin compounds were very simple and comprehensive and considered to be enough for identification as will be described. Accordingly, the authors used mixture A as standards for qualitative analysis without further purification. However, the reaction of a mixture of BDMeTC and DBMeTC (mixture A) with Grignard's reagents having bulky groups such as *sec*BuMgBr or EHMgBr gave very low yields of products, considered from the relative peak area ratios of target products to the other products, especially for derivatives of BDMeTC in a lower yield. As an example, the complete mass spectra of BEHDMeT could not be obtained. In this case, a mass fragment by GC/MS/SIM (*m/z* 263, M − 57, EHMe$_2$Sn$^+$) was used for the identification of products formed in combination with the RT of GC/MIP/AED.

Identification and Determination of Organotin Compounds in a Sediment and a Technical TBTC. Organotin compounds added to Kanto loam soil were quantitatively recovered except for MBTC. The reason the recovery of MBTC was low (37%) appears to be through low extraction efficiency of MBTC with tropolone/benzene because organotin compounds including MBTC spiked to HCl-treated silica gel column were quantitatively recovered.

A whole gas chromatogram on HP-1 obtained from a sediment sample collected on August 20, 1992, in Aburatsubo marina, is shown in Figure 2A. Three large peaks [RTs (retention times) = 3.550, 5.256, and 6.495 min] and one small peak appearing at ∼10 min were assigned easily to *n*-butyltin trichloride (MBTC), di-*n*-butyltin dichloride (DBTC), tri-*n*-butyltin chloride (TBTC), and triphenyltin chloride (TPTC), respectively, by comparing RTs with those of authentic standards. The other small peaks observed at RT 5−7.0 and 7.0−8.2 min are magnified in Figure 2B,C, respectively. The peaks on the gas chromatogram, which were characterized as organotin compounds by an emission spectra method (Łobiński et al., 1992; Suzuki et al., 1994; Yamamoto et al., 1997), were numbered in the order of short to long.

DBTC and TBTC in the sediment extract were always accompanied by a pair of peak numbers, 1 and 2 and 8 and 9, respectively, in front of their great peaks as shown in Figure 2B, although the levels were low. These products have also been consistently found even in working standard solutions when high concentrations were employed. Accordingly, there is little doubt that these chemicals originate from contaminants in TBTC. Consequently, a commercially available TBTC of technical grade (95%) was investigated to elucidate their structures, and the GC/MIP/AED spectrum of its methylated product is shown in Figure 2D.

RTs of peaks 1, 2, 8, and 9 in the technical TBTC coincided with those of the methylated products in the sediment sample. Ion chromatograms (GC/MS) of peaks 8 and 9 in the technical TBTC using isotopic ions clusters at *m/z* 249 (M − 57), 193 (BuMeHSn$^+$), and 135 (MeSn$^+$) were characteristic of TBTC. The result shows that these three compounds are structurally related and thought to be isomers. Mass spectra at these three peaks gave almost the same patterns and were indistinguishable from each other (data not shown). Accordingly, it was thought that these products were formed by replacement of one *n*-butyl group in TBTC by one of the isomers of the butyl group other than the *n*-butyl group. Among three isomers considered, the bulkiest isomer (the *tert*-butyl group) is not likely to be able to approach easily on the tin atom at the center of DBTC molecule, and therefore this group was put outside the scope of the present consideration. Reaction products of mixture A with isoBuMgBr or *sec*BuMgBr were compared with those of methyl derivatives of a technical grade of TBTC on HP-1 and DB-5 using GC/MIP/AED. The RTs of peaks 8 and 9 in both panels B and D of Figure 2 coincided with those of authentic DBisoBMeT and DB*sec*BMeT, respectively, and the RTs of peaks 1 and 2 (Figure 2B) coincided with those of authentic BisoBDMeT and B*sec*BDMeT, respectively, on HP-1 and DB-

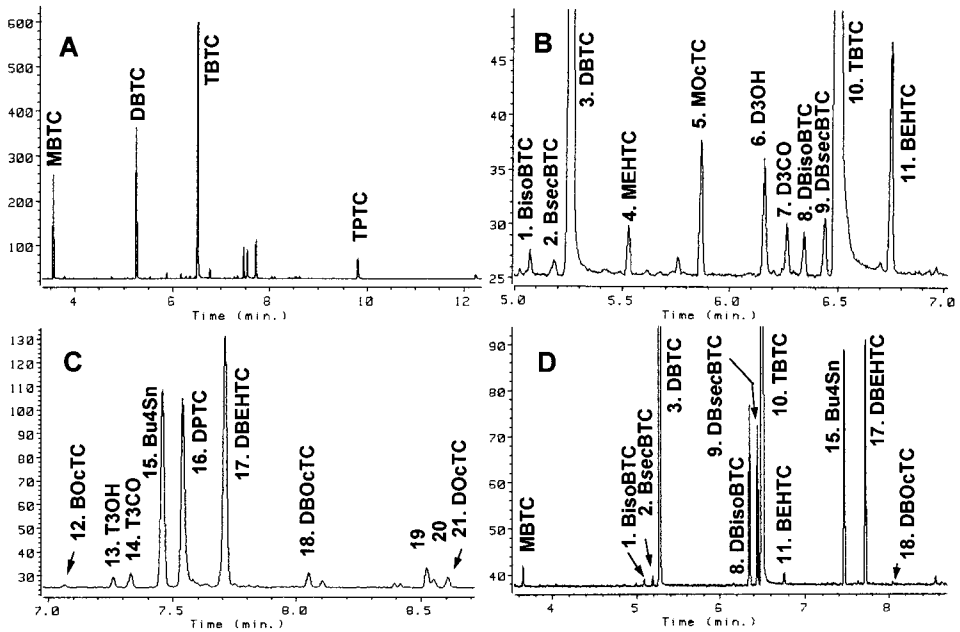

Figure 2. GC/MIP/AED chromatograms obtained from the sediment extract (August 20, 1992) at a marina in Aburatsubo Bay (A, whole chromatogram; B, 5−7 min (enlargement of panel A); C, 7−8.7 min (enlargement of panel A) and from a commercially available technical tri-*n*-butyltin chloride (D).

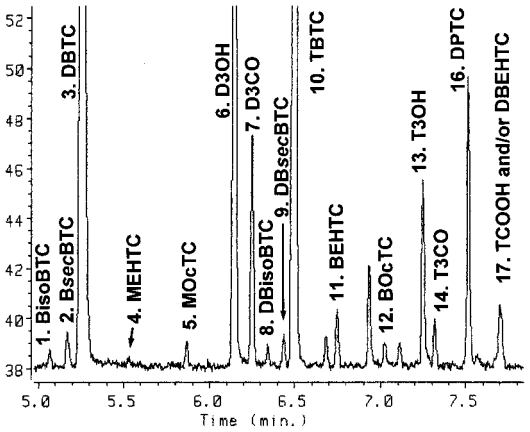

Figure 3. GC/MIP/AED chromatogram obtained from the top-shell collected in a Tokyo market (1991) on HP-1.

5. DBTC and TBTC in fish or shellfish extracts were also always accompanied by pairs of peaks, 1 and 2 and 8 and 9, respectively, in front of their peaks as exemplified by the gas chromatogram of top-shell extract in Figure 3. These peaks were considered to be derived from halogenated iso- and *sec*-butyl compounds, which might be contaminated at the first step of the industrial production of TBTC.

Peak 5 in the sediment sample (Figure 2B) was supposed to be *n*-octyltin trichloride (MOcTC) on the basis of the RTs on HP-1, DB-5, and DB-225, and this assignment was further confirmed by the use of GC/MS/SIM (Figure 4A). An ions cluster at *m*/*z* 263 suggests the presence of a group, $OcMe_2Sn^+$. The presence of this chemical in sediment samples has been previously reported by Chau et al. (1997). This compound was also found in the top-shell extract, although the level was low (Figure 3, peak 5), and the origin of this compound may be in the use of DOcTC, which is used as a stabilizer for packing materials or poly(vinyl chloride) (PVC).

Two couples of peaks 6 and 7 and 13 and 14 in the sediment sample (Figure 2B,C) and in the top-shell sample (Figure 3) were easily assigned to *n*-butyl(3-hydroxybutyl)tin dichloride (D3OH) and *n*-butyl(3-oxobutyl)tin dichloride (D3CO) and di-*n*-butyl(3-hydroxybutyl)tin chloride (T3OH) and di-*n*-butyl(3-oxobuty)tin chloride (T3CO), respectively, on the basis of RTs on HP-1. These assignments in the sediment sample were also supported by the use of three other columns, DB-5, DB-225, and DB-1701, and by the results of GC/MS/SIM (Figure 4B−E). The presence of these compounds in the top-shell was already shown by GC/MS/SIM (Suzuki et al., 1992). These organotin compounds have been found invariably in pairs in some species of natural fish flesh and shellfish (Suzuki et al., 1992, 1994, 1998), natural fish liver (Suzuki et al., 1992), natural fish eggs (Suzuki et al., 1992), fish liver experimentally exposed to TBTO (Yamamoto et al., 1997), mammals experimentally exposed to TBTC (Matsuda et al., 1993; Ueno et al., 1997), and seawater in a marina (Suzuki et al., 1996). The presence of these oxygenated compounds in sediment samples clearly supports the idea reported previously that the biological degradation of TBTC in seawater actually occurs (Suzuki et al., 1996).

Peak 15, the first peak of three relatively large peaks around 7.6 min in the sediment sample, was easily characterized as tetrabutyltin (Bu4Sn) on the basis of RTs on HP-1 and DB-5, and the main mass fragment ions [*m*/*z* 291 (M − 57, Bu_3Sn^+), 235 (M − 113, Bu_2HSn^+), and 179 (BuH_2Sn^+)] were also identical with those of the authentic sample as shown in Figure 4F. The presence of this compound in sediment samples was already published by Chau et al. (1997). It became apparent that the technical TBTC also contains 0.5% of this chemical as shown in Figure 2D. This fact suggests two possibilities: that Bu4Sn in the sediment sample originates from the impurities in technical TBTC or TBTO or that the antifouling paints used around the marina were, at least in part, not of polymer type.

Peak 16 in the sediment sample (Figure 2C) corresponded to DPTC in its RTs on HP-1 and DB-5. TPTC was a chemical species which had been mainly used from the late 1980s to early 1990s in place of TBTO and

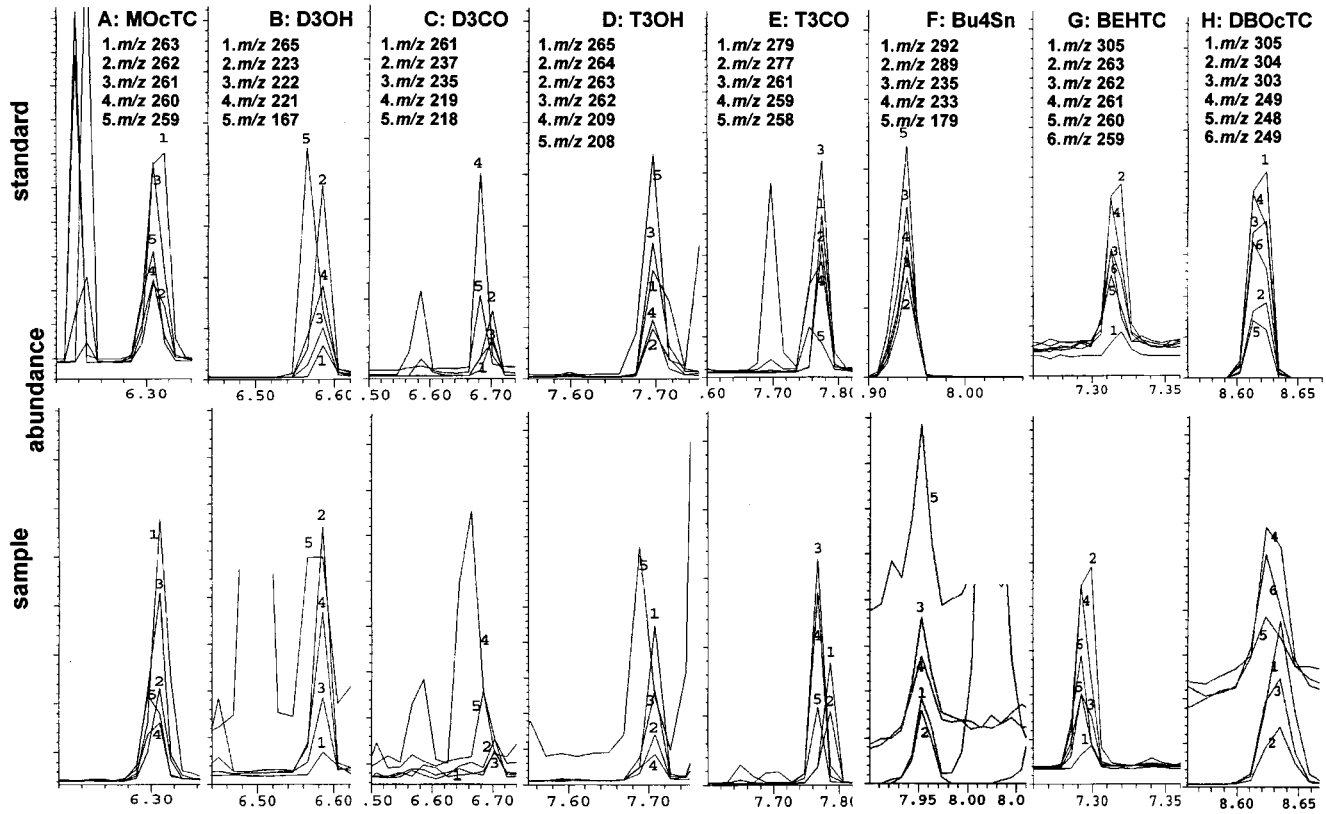

Figure 4. Identification of organotin compounds in a sediment sample (A−F and H) and in a technical TBTC (G) by GC/MS/SIM in comparison with methylated authentic standards.

often found in fish samples in Japan (Ishizaka et al., 1989b). However, because of its high bioconcentration factor, actual uses have been prohibited since the early 1990s. It is no wonder that DPTC, which is a degradation product of TPTC, was found in the sediment. Ultimately, the structure of compound **16** was confirmed by GC/MS/SIM (data not shown).

The studies with the fragmentations of tetraalkylated organotin compounds on GC/MS should be mentioned here because exact interpretation of GC/MS spectra provides a better understanding and explanation of the work carried out and the results obtained. A series of measurements of organotin compounds in our laboratory has shown that they are following several rules between chemical structures of methylated simple alkyltin compounds (straight-chain compounds), $R_aR_bR_c$-SnMe ($R_a \geq R_b \geq R_c >$ Me) and their mass spectral fragmentations in an electron impact mode: (1) Molecular ion is not observed. (2) A weak *m/z* M − 15 (Me) ion cluster (1−2% of a base peak or less) is consistently observed. (3) Ion clusters, M − R_a, M − R_b, and M − R_c are also observed, but their intensities were in a widespread range (5−100% of a base peak). When $R_a = R_b$, their relative intensities were M − R_a (= M − R_b) > M − R_c. (4) R_aMeHSn$^+$, R_bMeHSn$^+$, and R_cMeHSn$^+$ are relatively strong (50−100% of a base peak). When $R_a = R_b$, their intensities were in the order of R_aMeHSn$^+$ (= R_bMeHSn$^+$) > R_cMeSnH$^+$. (5) R_aSn$^+$, R_bSn$^+$, and R_c-Sn$^+$ are observed, although their intensities are weak (5−10%), but *m/z* 135 (MeSn$^+$) is relatively strong by overlapping with one of isotope peaks of MeH_2Sn^+ (*m/z* 137)(>20%) and can sometimes be the strongest peak. (6) Unlike $R_aR_bR_c$MeSn, R_aR_bMe$_2$Sn derivatives show the strongest fragment ion cluster at *m/z* 151 due to Me$_2$-HSn$^+$ and give relatively strong M − R_a and M − R_b ion clusters due to R_bMe$_2$Sn$^+$ and R_aMe$_2$Sn$^+$ (30−100%), and their order is generally R_aMe$_2$Sn$^+$ > R_bMe$_2$Sn$^+$. R_a-MeHSn$^+$ and R_bMeHSn$^+$ were generally weak (∼15% or less). Ion cluster M − 15 also raises intensity >2-fold by increased incidence of fragmentation (2−12%). (7) An ion cluster, *m/z* 121, with relatively weak intensity due to HSn$^+$ is consistently observed for any tin compound (8−15%). (8) R_aMe$_3$Sn derivatives give very strong fragment ion clusters of almost equal intensities at *m/z* 151 (Me$_2$HSn$^+$) and 165 (Me$_3$Sn$^+$), and M − 15 (R_aMe$_2$Sn$^+$) is also moderately strong (40−50%). (9) When a mass spectrum of an organotin compound is measured, care must be paid in the selection of RT where the mass spectrum is taken, because a total ion peak observed is a mixture composed of 10 stable isotopes of tin, each isotope giving a different RT for each compound; that is, selection of a different RT gives a different mass spectrum on a total ion peak (Suzuki et al., 1992). This can be also easily understood from SIM spectra in Figure 4. Accordingly, the mass spectrum was principally measured at a maximum of each peak.

Peak 17 in the sediment extract in Figure 2C, the highest peak of the triplet, showed very similar chromatographic behavior to that of TCOOH on the HP-1 and DB-5 columns, but the behavior on the DB-225 and DB-1701 columns could completely differentiate it from TCOOH. Its mass spectrum is shown in Figure 5A, and the mass spectral pattern and the RTs on the HP-1 and DB-5 columns were identical with those of compound **17** in the technical TBTC (the mass spectrum of **17** in the technical TBTC is not shown). This shows mass fragment ion clusters at *m/z* 347, 305, 249, 233, 193 (base peak), 137, and 121. As a result of careful examination of these fragments by GC/MS/SIM, DB-OcTC was proposed as a plausible structure of **17**. However, the newly synthesized DBMeOcT was not in

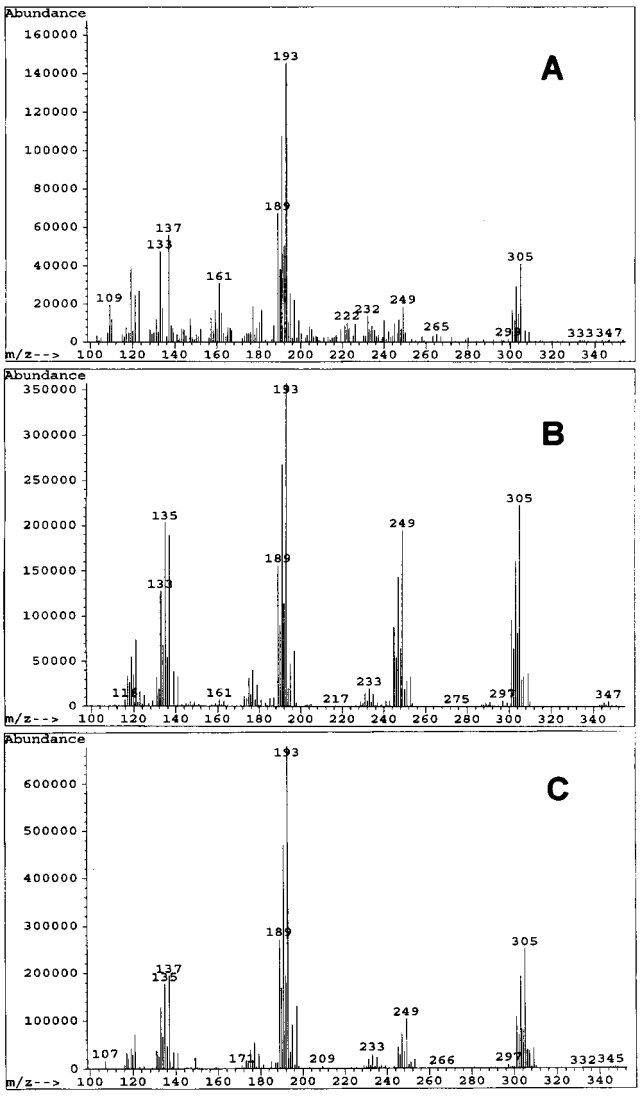

Figure 5. GC/MS spectra of **17** in sediment sample (A), authentic di-*n*-butyl methyloctyltin (DBMeOcT, B), and authentic di-*n*-butyl(2-ethylhexyl)methyltin (DBEHMeT, C).

agreement with **17** in the sediment or in the technical TBTC, but, to our surprise, the RT of synthesized methylated DBOcTC (DBMeOcT) agreed with that of **18** in the sediment sample (Figure 2C). The identity of **18** in the sediment sample with DBOcTC was further confirmed by GC/MS/SIM (Figure 4H). In addition to this, the presence of DBOcTC in the technical TBTC was also validated on the HP-1 (Figure 2D, peak 17) and DB-5 columns by GC/MIP/AED and by GC/MS/SIM (data not shown). As might be expected, the authentic specimen of DBOcTC (Figure 5B) showed a mass spectral pattern very similar to that of peak 17 in Figure 2C (Figure 5A). A more decisive difference was that it had a different RT on GC/MIP/AED. On the basis of these results, we speculated that compounds **17** and **18** are structurally related and probably structural isomers of each other. The next candidate was a compound with an isomer of the *n*-octyl group, 2-ethylhexyl group. The 2-ethylhexyl group has been often used as a substituent in industrial chemicals, for example, di(2-ethylhexyl)-phthalate and di(2-ethylhexyl)adipate. The reaction of mixture A with EHMgBr gave a mixture of di-*n*-butyl-(2-ethylhexyl)methyltin (DBEHMeT) and *n*-butyl(2-ethylhexyl)dimethyltin (BEHDMeT). The mass spectrum of the former product shown in Figure 5C was in substantial agreement with the mass spectrum of compound **17** in the sediment sample (Figure 5A) and also with that in the technical TBTC (data not shown). The presence of DBEHTC in this top-shell sample is not clear on the chromatogram in Figure 3 because of overlapping of DBEHTC with TCOOH on the HP-1 column. However, DBEHTC was separated from TCOOH using the DB-225 column. We reported that this compound was concentrated with time in transplanted marine mussel, *Mytilus graynus*, from a lightly polluted area to a heavily polluted area (the same sampling site as that of the sediment sample in the present study) and the structures of TBTC metabolites were already determined, but the structure of compound **17** has not yet been determined (Suzuki et al., 1998). To our knowledge, this is the first report of the occurrence of DBEHTC in sediment or marine samples.

Peak 11 in the sediment sample (Figure 2B) showed an RT very similar to that of *n*-butyl(3-carboxypropyl)-tin dichloride (DCOOH) on the HP-1 column, but it was slightly longer than that of DCOOH. RTs of this product completely coincided with those of compound **11** in the technical TBTC on the HP-1 (Figure 2D) and DB-5 columns, and therefore it was concluded that they must be the same. Because the complete mass spectrum of **11** in the sediment sample was not measured because of interferences at a low mass field, only the presence of ion clusters due to isotope peaks at *m*/*z* 263 and 305 was confirmed. As a result, our efforts have been focused on elucidation of the structure of **11** in the technical TBTC in place of one in the sediment sample. According to the general rule of mass fragmentation described above, *m*/*z* 305 of **11** should be M − 15 and the molecular weight should be 320. The presence of *m*/*z* 263 strongly suggests the presence of an $OcMe_2Sn$ group in the molecule. On the other hand, DCOOH does not show an ion cluster at *m*/*z* 263. Taken together, these observations appear to provide persuasive evidence for the possible involvement of *n*-butyl-*n*-octyltin dichloride (BOcTC). On the other hand, one of two reaction products of mixture A with OcMgBr (which has a shorter RT on GC/MIP/AED and is a minor component comprising 8% of reaction products), namely BDMeOcT, gave ion clusters at *m*/*z* 305 and 263 on the mass spectrum, as might be expected. However, the RT on HP-1 (GC/MIP/AED) of BDMeOcT gave a rather longer RT, which corresponded to compound **12** rather than to **11** of the sediment extract (Figure 2C). These results suggest that the relationship between **11** in the sediment sample and BOcTC is similar to that between DBEHTC and DBOcTC. Reaction of mixture A with EHMgBr gave DBEHMeT (74%) predominantly and only a small amount of BEHDMeT (1%), and therefore a complete mass spectrum of the latter was not obtained. However, the results of GC/MIP/AED and GC/MS/SIM on the HP-1 (Figure 4G) and DB-5 columns (data not shown) clearly showed that the assignment of **11** in the technical TBTC and in the sediment sample to *n*-butyl 2-ehtylhexyltin dichloride (BEHTC) was correct. The presence of BEHTC was also identified in the top-shell extract (Figure 3, peak 11). This fact also strongly suggests the possibility that **17** in the top-shell extract is DBEHTC. Probably, BEHTC in the top-shell extract would be produced by metabolism of DBEHTC absorbed. The presence of BOcTC was also confirmed in the top-shell sample, although its level was very low (Figure 3, peak 12).

Table 4. Organotin Compounds in a Technical Grade of TBTC

compound	%[a]	compound	%[a]
MBTC	0.06	TBTC	96.0
BisoBTC	0.02	BEHTC	0.03
B*sec*BTC	0.03	Bu4Sn	0.5
DBTC	2.1	DBEHTC	0.5
DBisoBTC	0.4	DBOcTC	0.007
DB*sec*BTC	0.3	others	0.03

[a] The results are expressed as a percentage of the sum of the total peak areas on HP-1.

Table 5. Organotin Compounds in a Sediment[a]

compound	level	compound	level	compound	level
MBTC	105	DB*sec*BTC	5[b]	T4OH	3
BisoBTC	2[b]	TBTC	720	DPTC	93
B*sec*BTC	1[b]	D4OH	10	TCOOH	ND
DBTC	307	DCOOH	ND	DBEHTC	98[b]
MEHTC	4	BEHTC	14[b]	DBOcTC	10[b]
MOcTC	12[b]	BOcTC	1[b]	DOcTC	6
D3OH	14	T3OH	5	TPTC	65
D3CO	8	T3CO	5		
DBisoBTC	4[b]	Bu4Sn	92		

[a] Concentrations (ppb on dry weight basis) of organotin species reported in this paper were not corrected for recovery. [b] Authentic standards of these compounds were not obtained in pure forms, and alkylation methods used for synthesis of these compounds are also different from those for the sample preparations of sediment extracts; therefore recovery tests could not be performed. The values listed were calculated depending on the assumption that recoveries, responsiveness in GC/MIP/AED, and yields in alkylation of alkyltin compounds were equal to those of TBTC.

The compound of peak 4 in the sediment sample was at a very low level and not found in the technical TBTC (Figure 2B). There was no compound corresponding to this RT among available samples, but, on the basis of the RT on the HP-1 column and the presence of DBEHTC and BEHTC in the sediment sample, a 2-ethylhexyl derivative was chosen as a powerful candidate. This peak was finally identified as methylated 2-ethylhexyltin trichloride (MEHTC) depending on the correspondence of this peak to that of the synthesized standard (MEHMeT) on the HP-1 and DB-5 columns. It was also made clear that MEHTC was present at a trace level in the top-shell sample (Figure 3, peak 4).

Peak 21 was identified as di-*n*-octyltin dichloride (DOcTC) by means of GC/MIP/AED. The presence of this chemical in the sediment sample was already reported by Chau et al. (1997).

The analytical results of organotin compounds in the technical TBTC and in the sediment sample are depicted in Tables 4 and 5. In the organotin compounds synthesized, some of the trialkyltin chlorides were obtained only as a mixture, and therefore recovery tests could not be performed. In addition to this, it was supposed that reaction yields of mixture A with Grignard's reagents are not always the same with those of organotin compounds in the sediment sample with MeMgBr, although the reaction products are the same. However, it is generally known that similar compounds respond similarly in plasma in the determination using GC/MIP/AED (Huang et al., 1990; Łobiński et al., 1992). This supports the possibility that peak area is proportional to tin concentration in a series of organotin compounds. Consequently, the results in Table 4 were defined in terms of Sn. On the other hand, measurements of organotin compounds in Table 5 were in part performed by simply comparing peak areas with that of TBMeT, on the assumption that TBMeT and a given compound have the same sensitivity and the same molecular weight (i.e., not corrected for molecular weights). Among organotin compounds found in the technical TBTC, the presence of iso- and *sec*-butyl isomers Bu4Sn, DBTC, and MBTC could be easily expected considering the industrial preparation process. However, it is not known why DBEHTC, DBOcTC, and their debutylated products would be found in the technical TBTC. It is of interest that the relative ratio among Bu4Sn, BEHTC, and DBOcTC in the technical TBTC is very similar to that in the sediment sample. It seems that the concurrent appearance of unexpected chemicals in a technical TBTC and in a sediment sample is more than coincidence, but the amount of DBEHTC in the technical TBTC is too little (0.5%) to say that DBEHTC was intentionally added. The reason DBEHTC was present in a technical TBTC remains to be ascertained.

Earlier experience in our laboratory has shown that the formation of derivatives in seawater in the summer season (July−September) were in the order D3OH > D4OH > D3CO in dibutyltin derivatives and T3OH > T4OH > T3CO in tributyltin derivatives (Suzuki et al., 1996). The amount of dibutyltin derivatives in the Aburatsubo sediment in the present study was in the same order with those in seawater, but the amount of tributyltin derivatives in sediment sample was slightly different and was in the order T3OH ≅ T3CO > T4OH but was in general agreement with those in seawater (Table 5). The relative ratio of these compounds in the sediment sample may reflect the biological degradation process in seawater and the sedimentation after adsorption onto particulate.

In conclusion, the presence of >20 organotin compounds was confirmed in a sediment collected in a marina and their structures were elucidated. First, chemicals such as TBTC or TPTC, which are the main active ingredients in antifouling paint, are derived from the direct sedimentation of organotin compounds released from boats or detachment of old paint in repainting. Second, another class of compounds is from the sedimentation of biological degradation products of tributyltin compounds in seawater, such as *n*-butyl(3-hydroxybutyltin) dichloride, *n*-butyl(3-oxobutyl)tin dichloride, a part of dibutyltin chloride, di-*n*-butyl(3-hydroxylbutyl)tin chloride, and di-*n*-butyl(3-oxobutyl)tin chloride. Third, these classes of compounds are from the contaminants in technical grade tributyltin compounds, and their formation can be easily expected from their preparation processes. Among these substances are a part of di-*n*-butyltin dichloride, butylisobutyltin dichloride, *n*-butyl-*sec*-butyltin dichloride, di-*n*-butylisobutyltin chloride, di-*n*-butyl-*sec*-butyltin chloride, and tetrabutyltin. Fourth, the last class of compounds is a class of chemicals that cannot be predicted from TBTC or TBTO preparation processes, and their sources are not specified. Di-*n*-butyl(2-ethylhexyl)tin chloride, *n*-butyl-(2-ethylhexyl)tin dichloride, and di-*n*-butyloctyltin chloride belong to this category. The contamination levels of butyl(2-ehtylhexyl)tin and Bu4Sn in the sediment sample were almost the same, and this relative ratio is also almost the same as that in a technical TBTC. It is not clear if this experimental result is a coincidence or the result of intentional addition, but it is of particular interest that almost all organotin compounds derived from various sources, such as technical TBTC, biological

degradation process of TBTC, and stabilizer in plasticware and PVC, were found in a sediment sample.

ABBREVIATIONS USED

TBTs, tributyltin compounds; GC/MIP/AED, gas chromatography/helium atomospheric pressure microwave induced plasma/atomic emission detection system; AED, atomic emission detector; Et_2O, diethyl ether; MeMgBr, methylmagnesium bromide; EtMgBr, ethylmagnesium bromide; isoBuMgBr, isobutylmagnesium bromide; *sec*BuMgBr, *sec*-butylmagnesium bromide; BuMgCl, *n*-butylmagnesium chloride; OcMgBr, *n*-octylmagnesium bromide; AcOH, acetic acid; EHMgBr, 2-ethylhexylmagnesium bromide; EtOAc, ethyl acetate; GC/MS, gas−liquid chromatography/mass spectrometry; GC/MS/SIM, gas−liquid chromatography/mass spectrometry/selective ion monitoring; RT, retention time; other abbreviations are given in Table 1 and Figure 1.

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LITERATURE CITED

Arnold, S. F.; Klotz, D. M.; Collins, B. M.; Vonier, P. M.; Guillette, L. J., Jr.; McLachlan, J. A. Synergistic activation of estrogen receptor with combinations of environmental chemicals. *Science* **1996a**, *272*, 1489.

Arnold, S. F.; Robinson, M. K.; Notides, A. C.; Guillette, L. J., Jr.; McLachlan J. A. A yeast estrogen screen for examining the relative exposure of cells to natural and xenoestrogens. *Environ. Health Perspect.* **1996b**, *104*, 544−548.

Bryan, G. W.; Gibbs, P. E.; Burt, G. R. A comparison of the effectiveness of tri-*n*-butyltin chloride and five other organotin compounds in promoting the development of imposex in the dog-whelk *Nucella lapillus*. *J. Mar. Biol. Assoc. U.K.* **1988**, *68*, 733−744.

Chau, Y. K.; Maguire, R. J.; Brown, M.; Yang, F.; Bachelor, S. P. Occurrence of organotin compounds in the Canadian aquatic environment five years after the regulation of antifouling uses of tributyltin. *Water Qual. Res. J. Can.* **1997**, *32*, 453−521.

Fish, R. H.; Kimmel, E. C.; Casida, J. E. Bioorganotin chemistry: Reactions of tributyltin derivatives with a cytochrome P-450 dependent monooxygenase enzyme system. *J. Organomet. Chem.* **1976**, *118*, 41−54.

Gibbs, P. E.; Bryan, G. W.; Pascoe, P. L.; Burt, G. R. The use of the dog-whelk, *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination. *J. Mar. Biol. Assoc. U.K.* **1987**, *67*, 507−523.

Hattori, Y.; Kobayashi, A.; Takemoto, S.; Takami, K.; Kuge, Y.; Sugimae, A.; Nakamoto, M. Determination of trialkyltin, dialkyltin, and triphenyltin compounds in environmental water and sediments. *J. Chromatogr.* **1984**, *315*, 341−349.

Huang, Y.; Ou, Q.; Yu, W. Study of gas chromatography-microwave-induced plasma atomic emission spectrometry. Part 1. Effect of the structure of a compound on the determination of its empirical formula. *J. Anal. At. Spectrom.* **1990**, *5*, 115−120.

Ishizaka, T.; Suzuki, T.; Saito, Y. Metabolism of dibutyltin dichloride in male rats. *J. Agric. Food Chem.* **1989a**, *37*, 1096−1101.

Ishizaka, T.; Nemoto, S.; Sasaki, K.; Suzuki, T.; Saito, Y. Simultaneous determination of tri-*n*-butyltin, di-*n*-butyltin, and triphenyltin compounds in marine products. *J. Agric. Food Chem.* **1989b**, *37*, 1523−1527.

Łobiński, R.; Dirkx, W. M. R.; Ceulemans, M.; Adams, F. C. Optimization of comprehensive speciation of organotin compounds in environmental samples by capillary gas chromatography helium microwave-induced plasma emission spectrometry. *Anal. Chem.* **1992**, *64*, 159−165.

Sasaki, K.; Suzuki, T.; Saito, Y. Determination of tri-*n*-butyltin and di-*n*-butyltin compounds in yellowtails. *Bull. Environ. Contam. Toxicol.* **1988**, *41*, 888−893.

Smith, B. S. Male characteristics in female *Nassarius obsoletus*: variations related to locality, season and year. *Veliger* **1981a**, *23*, 212−216.

Smith, B. S. Reproductive anomalies in stenoglossan snails related to pollution from marinas. *J. Appl. Toxicol.* **1981b**, *1*, 15−21

Smith, B. S. Male characteristics on female mud snails caused by antifouling bottom paints. *J. Appl. Toxicol.* **1981c**, *1*, 22−25.

Smith, B. S. Tributyltin compounds induce male characteristics on female mud snails *Nassarius obsoletus* = *Ilyanassa obsoleta*. *J. Appl. Toxicol.* **1981d**, *1*, 141−144.

Stang, P. M.; Seligman, P. F. Distribution and fate of butyltin compounds in the sediment of San Diego Bay. In *Proceedings of the Organotin Symposium, Oceans '86*, Washington, DC, 1986; IEEE: New York, 1986; Vol. 4, pp 1256−1261.

Suzuki, T.; Matsuda, R.; Saito, Y. Molecular Species of Tri-*n*-butyltin Compounds in Marine Products. *J. Agric. Food Chem.* **1992**, *40*, 1437−1443.

Suzuki, T.; Matsuda, R.; Saito, Y.; Yamada, H. Application of helium microwave-induced plasma emission detection system to analysis of organotin compounds in biological samples. *J. Agric. Food Chem.* **1994**, *42*, 216−220.

Suzuki, T.; Yamada, H.; Yamamoto, I.; Nishimura, K.; Kondo, K.; Murayama, M.; Uchiyama M. Chemical species of organotin compounds in seawater and their seasonal variations. *J. Agric. Food Chem.* **1996**, *44*, 3989−3995.

Suzuki, T.; Yamamoto, I.; Yamada, H.; Kaniwa, N.; Kondo, K.; Murayama, M. Accumulation, Metabolism, and Depuration of Organotin Compounds in the Marine Mussels *Mytilus graynus* and *Mytilus edulis* under Natural Coditions. *J. Agric. Food Chem.* **1998**, *46*, 304−313.

Ueno, S.; Suzuki, T.; Susa, N.; Furukawa, Y.; Sugiyama, M. Effect of SKF-525A on liver metabolism and hepatotoxicity of tri-and dibutyltin compounds in mice. *Arch. Toxicol.* **1997**, *71*, 513−518.

Unger, M. A.; Macintyre, W. G.; Huggett, R. J. Sorption behavior of tributyltin on estuarine and freshwater sediments. *Environ. Toxicol. Chem.* **1988**, *7*, 907−915.

Valkirs, A. O.; Seligman, P. F.; Lee, R. F. Butyltin partitioning in marine waters and sediments. In *Proceedings of the Organotin Symposium, Oceans '86*, Washington, DC, 1986; IEEE: New York, 1986; Vol. 4, pp 1165−1170.

Vonier, P. M.; Crain, D. A.; McLachlan, J. A.; Guillette, L. J., Jr.; Arnold, S. F. Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. *Environ. Health Perspect.* **1996**, *104*, 1318−1322.

Waldock, M. J.; Thain, J. E.; Waite, M. E. The Distribution and potential toxic effects of TBT in UK estuaries during 1986. *Appl. Organomet. Chem.* **1987**, *1*, 287−301.

Yamamoto, I.; Nishimura, K.; Suzuki, T.; Takagi, K.; Yamada, H.; Kondo, K.; Murayama, M. Accumulation, transformation, and elimination of bis(tri-*n*-butyltin) oxide in red sea bream, *Pagrus major*, under laboratory conditions. *J. Agric. Food Chem.* **1997**, *45*, 1437−1446.

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