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Distribution of antifouling biocides in a coastal area of Tanabe Bay, Japan

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Abstract

Tributyltin (TBT) and triphenyltin (TPT) concentrations in water samples from Tanabe Bay were found to range from 4–28 ng l^{-1} and 3–7 ng l^{-1}, respectively. In fishing ports, the concentrations of TBT in surface water were similar to those in bottom water. However, in aquafarming areas with poor flushing, the concentrations of TBT in bottom water were higher than those in surface water. This suggested that the TBT in water samples is re-eluted from sediment. No difference in the concentration of TPT was observed between the surface and bottom waters. The concentrations of TBT and TPT in sediment samples ranged from 3–23 μg kg^{-1} dry weight and 2–37 μg kg^{-1} dry weight. TBT and TPT concentrations ranged from 3.1–100 μg kg^{-1} and 3.1–7.2 μg kg^{-1} in oysters and gastropods, and from 1.1–4.9 μg kg^{-1} and $<$0.2–3.9 μg kg^{-1} in fish, respectively. Organotin concentrations in biota were lower than the tolerable average residue levels (TARLs). Alternative biocides – i.e. diuron, chlorothalonil, dichlofluanid, irgarol 1051 and Sea-Nine 211 – were also detected in surface water, and chlorothalonil and irgarol 1051 were detected in sediment. The concentrations of these compounds in surface water and sediment were lower than those reported previously. Dichlofluanid, chlorotharonil and irgarol 1051 were also found at low levels in oysters and gastropods, and at ranges of 325–339 μg kg^{-1}, 268–291 μg kg^{-1} and 43–49 μg kg^{-1}, respectively, in fish; the concentrations in fish were close to the TARL levels.

Introduction

Organotin (OT) compounds have been used as antifouling biocides since 1960. It is well known that OTs have adverse effects on marine organisms, causing such conditions as imposex in gastropods and shell-thickening in oysters (Gibbs & Bryan, 1986; Batley *et al.*, 1989; Gibbs *et al.*, 1991). In the 1980s, the use of the OT compound tributyltin (TBT) was regulated in several advanced countries, including England, France and the USA. In Japan, the use of bis (tributyltin) oxide (TBTO) was banned in 1990, and the use of 7 TBT species and 13 triphenyltin (TPT) species was controlled under the Law Concerning the Examination and Regulation of Manufacture, Etc. of Chemical Substances. However, in spite of these regulations in individual countries, no drastic decreases in the concentrations of TBT in aquatic environments were observed. For example, in the 1990s the TBT concentrations in water samples from the harbours and dockyards around San Diego Bay, Chesapeake Bay and Chinhae Bay often exceeded 100 ng l^{-1} (Evans & Huggett, 1991; Virkirs *et al.*, 1991; Law *et al.*, 1994; Shim *et al.*, 1998). And in the last two decades, the TBT concentrations detected in biological samples in various countries ranged from 10–100 μg kg^{-1} wet weight (ww) (Midorikawa *et al.*, 2004; Langston *et al.*, 2012). In Japan, these compounds have been detected in water, sediment and biota from harbours, marinas and estuaries, and particularly higher concentrations were measured in areas where boat activity was high and water movement was restricted (Harino *et al.*, 1998, 2003; Takahashi *et al.*, 1999). Moreover, the TBT and TPT concentrations detected in fish from the Port of Osaka, Japan were near the maximum acceptable concentrations in fish (Harino *et al.*, 2000).

Because TBT concentrations over the levels that caused imposex in gastropods were detected in various coastal areas around the world, in October 2001 the International Maritime Organization (IMO) adopted the Internal Convention on the Control of Harmful Anti-fouling Systems on ships (AFS Convention), which prohibited the use of OTs as active ingredients in antifouling systems for ships. Following these international restrictions on the application of OT-based antifoulants, paint manufacturers developed numerous alternative products which have been widely adopted by many shipping companies. As a result, it has been reported that higher concentrations of some alternative biocides, such as Sea-Nine 211, diuron, irgarol 1051, chlorothalonil and dichlofluanid, have been detected in the water environments of typical bays around the world (Harino, 2004). These monitoring surveys were carried out in typical bays in each country, and there have been few papers on monitoring surveys done in rural bays such as Tanabe Bay, where the present study was conducted. Tanabe Bay is a rural bay with many fishing ports, and it has been reported that Tanabe Bay was heavily contaminated by OTs 20 years ago (Kobayashi *et al.*, 2008). We considered that it would be helpful to clarify the current status of contamination by OTs and alternative biocides in water, sediment, mussels and fish collected from Tanabe Bay. The objective of this study was thus to

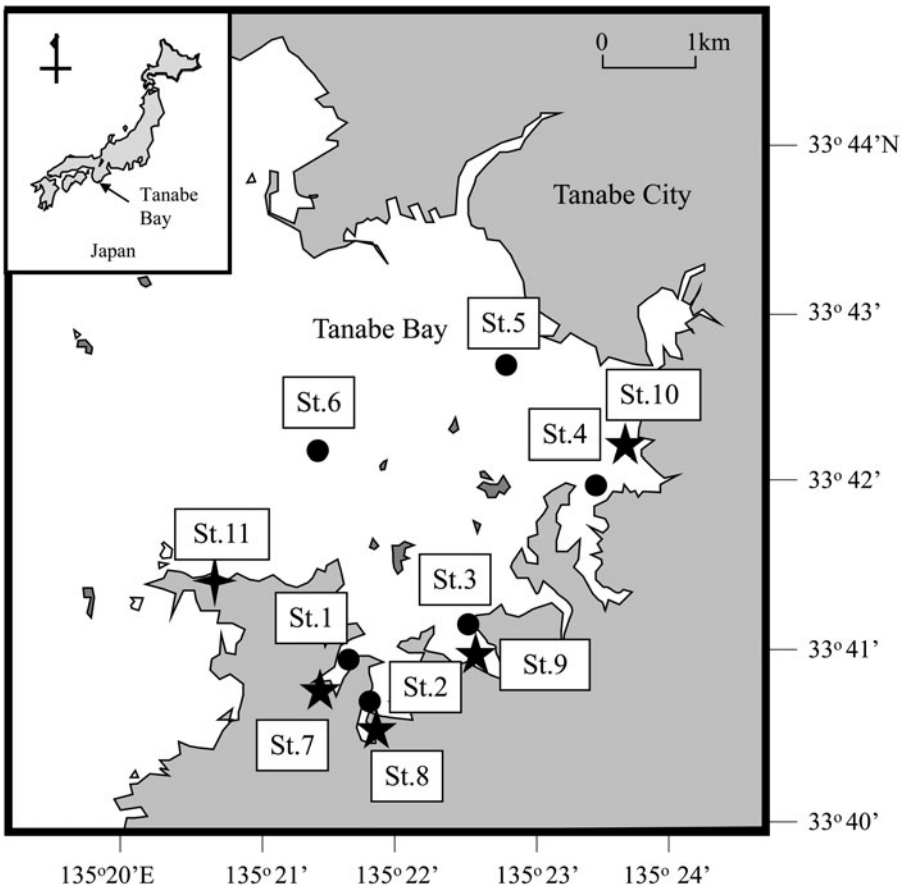

Fig. 1. Map of the study areas. ● water, sediment samples ★ mussel samples ♦ fish samples.

clarify the current status of contamination by antifouling biocides in the aquatic environment in Tanabe Bay and to assess the effect of the 2001 AFS Convention on rural bays.

Materials and methods

Sample collection

Tanabe Bay is located in the southern part of Japan. It has an area of 17.95 km^2, and the mouth of the bay is 4.05 km in length. The average concentrations of chemical oxygen demand and dissolved oxygen in the seawater of the bay are about 2 and 7 $mg\,l^{-1}$, respectively, indicating relatively clean water quality (Ministry of Environment 2011). There are many aquaculture facilities in this bay, and it is a treasure trove of biodiversity because the coastal areas are affected by the Japan Current. The sampling locations in Tanabe Bay are shown in Figure 1, and information regarding each sampling site is shown in Table 1. Water and sediment samples were collected at Stations 1–6 using buckets and Eckmanbirge grab samplers, and mussel and fish samples were taken at Stations 7–10 and Station 11, respectively, on 1–2 August 2016 (Figure 1). Water samples were stored at 3°C after being collected and were analysed within 3 days. Sediment and biological samples were stored at −20°C until analysis.

Chemical analysis

Organotin compounds

One litre of water from the samples was placed in a separation funnel, and 50 µl of mixed acetone solution containing 1 µg ml^{-1} each of monobutyltin trichloride ($MBTCl_3$)-d_9, dibutyltin dichloride ($DBTCl_2$)-d_{18}, tributyltin monochloride (TBTCl)-d_{27}, monophenyltin trichloride ($MPTCl_3$)-d_5, diphenyltin dichloride ($DPTCl_2$)-d_{10} and triphenyltin monochloride (TPTCl)-d_{15} as a surrogate standard, 5 ml of acetic acid-sodium acetate buffer (pH 5.0) and 1 ml of 5% $NaBEt_4$ were added to the separation funnel. After ethylation by shaking for 30 min, the analytes were extracted twice with 50 ml of hexane, and the organic layer was combined. After being concentrated to 1 ml by a rotary evaporator, 50 µl of mixed acetone solution containing 1 µg ml^{-1} each of tetrabutyltin (TeBT)-d_{36} and tetraphenyltin (TePT)-d_{20} as an internal standard was added to the organic solution. The final solution was concentrated to 0.5 ml in a nitrogen atmosphere. The analytes were determined by gas chromatography/mass spectrometry (GC/MS).

The method used to determine the OTs in the sediment and biological samples was based on that of Midorikawa *et al.* (2004) with some modifications. As a surrogate standard, 100 µl of mixed acetone solution containing 1 µg ml^{-1} each of $MBTCl_3$-d_9, $DBTCl_2$-d_{18}, TBTCl-d_{27}, $MPTCl_3$-d_5, $DPTCl_2$-d_{10} and TPTCl-d_{15} was added to a centrifuge tube containing 1 g of sediment or a biological sample. The analytes were extracted twice by shaking for 10 min with 10 ml of 1 M HCl – methanol/ethyl acetate (1/1). The combined supernatants and 30 ml of saturated NaCl solution were transferred to a separation funnel. The analytes were extracted twice with 15 ml of ethyl acetate/hexane (3/2) solution, and the organic layer was combined. Fifty ml of hexane was mixed into the organic layer and left to stand for 20 min. After removal of the aqueous layer, the organic layer was dried with anhydrous Na_2SO_4 and was concentrated to a trace level. The analytes were diluted with 5 ml of ethanol, 5 ml of acetic acid-sodium acetate buffer (pH 5.0), and 10 ml of distilled water, and were then ethylated by shaking with 1 ml of 5% $NaBEt_4$ for 30 min. The solution containing ethylated OTs was saponificated with 10 ml of 1 M KOH – ethanol solution by shaking for 1 h. Forty ml of distilled water and 10 ml of hexane

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Table 1. Information on the sampling sites in Tanabe Bay

Sample No.	Location	Sample	Water depth	Characterization
Station 1	N33.41.2004 E135.21.4725	Water, sediment	10 m	Fishing basin
Station 2	N33.40.8693 E135.21.7071	Water, sediment	10 m	Fishing basin, aquafarming
Station 3	N33.41.5288 E135.22.2026	Water, sediment	13 m	Aquafarming, poor flushing
Station 4	N33.42.2668 E135.23.2839	Water, sediment	11 m	Small boatslip
Station 5	N33.42.8005 E135.22.6596	Water, sediment	8 m	Boatslip
Station 6	N33.42.4454 E135.21.3615	Sediment	27 m	Offing of Tanabe Bay
Station 7	N33.68.3278 E135.35.3872	Mussel	–	Berthing of small boats
Station 8	N33.68.0665 E135.36.4954	Mussel	–	Small boatslip, aquafarming
Station 9	N33.68.4847 E135.37.4050	Mussel	–	Boatslip, aquafarming
Station 10	N33.70.5901 E135.39.2782	Mussel	–	Intertidal zone
Station 11	N33.68.7377 E135.34.1813	Fish	–	Sailing of small boats

were added to the solution, and the mixture was shaken for 10 min. The ethylated OT residue in the aqueous layer was extracted again by shaking for 10 min with 10 ml of hexane. The combined organic layers were dried with anhydrous Na_2SO_4. After being concentrated to 1 ml, the solution was cleaned with a Sep-Pak Florisil column (Waters Association). The Sep-Pak Florisil cartridge used for clean-up was prewashed with 10 ml of hexane. The hexane solution containing the analytes and then 10 ml of 5% diethyl ether/hexane solution were passed through the prewashed cartridge. All eluting solvent was collected in a bottom flask. The solution was concentrated to 0.5 ml after the addition of 100 µl of mixed acetone solution containing 1 µg ml^{-1} each of TeBT-d_{36} and TePT-d_{20} as an internal standard. The final solution was concentrated to 1 ml. The analytes were determined by GC/MS.

A Hewlett-Packard 6890 series gas chromatograph equipped with a mass spectrometer (5973 N) was used for analysis of the OTs. The separation was carried out in a capillary column coated with 5% phenyl methyl silicone (J&W Scientific; 30 m length × 0.25 mm i.d., 0.25 µm film thickness). The column temperature was held at 60°C for the first 2 min, then increased to 130°C at 20°C min^{-1}, to 210°C at 10°C min^{-1}, to 260°C at 5°C min^{-1}, and to 300°C at 10°C min^{-1}. Finally, the column temperature was held at 300°C for 2 min. The interface temperature, ion source temperature and ion energy were 28, 230°C and 70 eV, respectively. Selected ion monitoring was performed under this programme. The monitoring ions of 235 (233) for MBT, 261 (263) for DBT, 263 (261) for TBT, 253 (255) for MPT, 303 (301) for DPT, and 351 (349) for TPT were used to quantify the concentrations of the OTs. The qualifier ions are shown in parentheses. One microlitre of the sample was injected using splitless injection. The concentrations of OTs in this study are expressed as Sn_4^+.

Alternative biocides

One litre of water from the samples was placed in a separation funnel, and analytes were extracted twice by shaking for 10 min with 50 ml of dichloromethane. After drying using anhydrous Na_2SO_4, the organic layer was concentrated by a rotary evaporator to 1 ml. Fifty microlitres of hexane solution containing 0.5 mg l^{-1} of atrazine-d5 as an internal standard was added to the organic layer, and the organic layer was concentrated to 0.5 ml in a nitrogen atmosphere. The analytes were determined by GC/MS.

The method used for the determination of alternative compounds in sediment and biological samples was based on that of Harino *et al.* (2005). One gram of sediment or of a biological sample was placed in the centrifuge tube with 10 ml of acetone. The mixture was shaken for 10 min by a mechanical shaker. After centrifugation for 10 min, the supernatant was removed. The analytes were re-extracted with 10 ml of acetone for 10 min, and the mixture was centrifuged. The combined supernatants were concentrated by a rotary evaporator to 5 ml. Forty-five millilitres of distilled water, 1 g of zinc acetate, and 0.5 g of celite were added, and the mixture was left to stand for 20 min. After filtration, the analytes were extracted two times with 10 ml of dichloromethane. The organic layer was dried by anhydrous Na_2SO_4 and concentrated by a rotary evaporator to 1 ml. One hundred microlitres of hexane solution containing 1 mg l^{-1} of atrazine-d_5 was added to the organic layer, and the organic layer was concentrated to 1 ml in a nitrogen atmosphere. The analytes were determined by GC/MS.

A Hewlett-Packard 6890 series gas chromatograph equipped with a mass spectrometer (5973 N) was used for analysis of the alternative biocides. The separation was carried out in a capillary column coated with 5% phenyl methyl silicone (J&W Scientific; 30 m length × 0.25 mm i.d., 0.25 µm film thickness). The column temperature was held at 60°C for the first 1 min, then increased to 200°C at 10°C min^{-1}, and to 280°C at 5°C min^{-1}. The interface temperature, ion source temperature and ion energy were 280, 230°C and 7 0 eV, respectively. Selected ion monitoring was performed under this programme. The monitoring ions of 169 (281) for Sea-Nine 211, 189 (159) for diuron, 224 (332) for dichlofluanid, 266 (264) for chlorothalonil, 253 (182) for irgarol 1051 and 213 (198) for M1 were used to quantify the concentrations of the booster biocides. The qualifier ions are shown in parentheses. One microlitre of the sample was injected using splitless injection.

Results and discussion

Evaluation of the analytical procedure

The quality of the data obtained by the analytical procedure applied to the OTs and alternative biocides was examined. The recovery rates of OTs are shown in Table 2. When a 1 l water sample was spiked with 1 µg of OTs, the recovery rates and relative standard deviations (RSDs) of the OTs ranged from 73–10% and 3.0–7.5%, respectively. When 2 g of sediment samples or 2 g of biological samples were spiked with 1 µg of OTs, the recovery rates of the OTs ranged from 95–117% and 95–110%, respectively. The RSDs ranged from 6.1–11% for the sediment samples and 5.1–12% for the biological samples. The recovery rates of alternative biocides are shown in Table 3. When 1 l of water sample was spiked with 1 µg of alternative biocides, the recovery rates

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Table 2. Recovery rates and relative standard deviations of organotin (OT) compounds in water, sediment and biological samples

			Recovery rates (%)					
	Amounts (l or g)	Amount spiked (μg)	MBT	DBT	TBT	MPT	DPT	TPT
Sea water	2	1	84	73	100	74	93	98
			(3.0)	(4.7)	(3.2)	(7.5)	(4.1)	(3.6)
Sediment	2	1	107	108	107	117	102	95
			(6.1)	(8.3)	(11)	(7.5)	(8.4)	(7.2)
Biota	2	1	98	101	95	110	95	98
			(12)	(10)	(5.1)	(10)	(9.3)	(11)

Table 3. Recovery rates and relative correlation coefficients of alternative biocides in water, sediment and biological samples

			Recovery rates (%)					
	Amounts (l or g)	Amount spiked (μg)	Sea-Nine 211	Diuron	Dichlofluanid	Chlorothalonil	Irgarol 1051	M1
Seawater	1	1	67	93	65	65	97	83
			(10)	(9.6)	(13)	(12)	(8.5)	(10)
Sediment	2	1	120	89	123	66	120	109
			(9.6)	(9.6)	(11)	(7.2)	(14)	(10)
Biota	2	1	99	89	67	60	60	71
			(3.4)	(5.5)	(6.8)	(9.8)	(5.2)	(4.8)

The numbers in parentheses show the relative standard deviation.

and RSDs of the alternative biocides ranged from 65–97% and 8.5–13%, respectively. When 2 g of sediment samples or biological samples was spiked with 1 μg of alternative biocides, the recovery rates of the alternative biocides ranged from 66–123% and 60–99%, respectively. The RSDs ranged from 7.2–14% for the sediment samples and 3.4–9.8% for the biological samples.

The detection limits were calculated using a signal-to-noise ratio of 3. The detection limits were as follows: for each OT and alternative compound in water samples, 0.1 ng l^{-1} and 1 ng l^{-1}, respectively; for each OT in the sediment and biological samples, 0.1 μg kg^{-1} dry weight (dw); and for each alternative compound in the sediment and biological samples, 0.5 μg kg^{-1} dw.

Concentrations of OT compounds in water and sediment samples

The concentrations of TBT in water samples taken at Stations 1–5 ranged from 4.4–28 ng l^{-1} (Figure 2). Since the establishment of the AFS Convention, TBT concentrations have been reported in water samples in some port areas. Gao *et al.* (2017) reported that the concentrations of TBT ranged from <0.35–393.35 ng l^{-1} in the Three Gorges Reservoir region, China, while Liu *et al.* (2011) reported that the concentrations of TBT ranged from 6.36–76.83 ng l^{-1} in seawater collected along the coast of Hsiao Liouciou Island, Taiwan. The levels of TBT in water samples from Tanabe Bay were lower than those values. However, despite the ban on the use of TBT, the TBT concentrations in water samples from Tanabe Bay were above 1 ng l^{-1}, which was the level reported to cause imposex in gastropods in seawater (Horiguchi *et al.*, 1995).

In regard to TPT, the concentrations in our water samples ranged from 3.0–7.5 ng l^{-1} (Figure 2), which was similar to the TPT concentrations of <0.26–11.25 ng l^{-1} in the Three Georges Reservoir region of China (Gao *et al.*, 2017) and 0.13–1.26 ng l^{-1} along the Croatian Adriatic coast (Furdek *et al.*, 2012).

The concentrations of butyltins (BTs) and phenyltins (PTs) in the sediment samples are shown in Figure 3. The concentrations of TBT in the sediment samples from Stations 1–6 ranged from 3.1–24 μg kg^{-1}, which was similar to the ranges reported elsewhere. Namely, the concentrations of TBT in sediment were <1–22 μg kg^{-1} dw in the coastal areas of Panama (Batista-Andrade *et al.*, 2018); 13–144 μg kg^{-1} dw in Pescadoes Beach, Brazil (de Oliveira *et al.*, 2010); 3.0–40, 2.0–41 and 3.1–27 μg kg^{-1} dw in Busan Bay, Ulsan Bay, and the Korean coast, respectively (Lee *et al.*, 2015); 1.6–3.4 and 0.267–3.3 μg kg^{-1} dw in the Futian mangrove wetlands and Shenzhen Bay, China, respectively (Deng *et al.*, 2015); 12.6–191.7 μg kg^{-1} dw in the semi-closed port of Gdynia (Radke *et al.*, 2012); 2.3–81.8 μg kg^{-1} dw in the Mediterranean and the Atlantic (Anastasiou *et al.*, 2016); and <0.25–1.16 μg kg^{-1} dw in rivers, lakes and coastal waters in France (Cavalheiro *et al.*, 2016).

The concentrations of TPT in sediment were also similar to previous reports. The TPT concentrations in sediment samples from Tanabe Bay ranged from 2.3–37 μg kg^{-1} dw, *vs* <0.137–1.4 μg kg^{-1} dw and 1.1–5.5 μg kg^{-1} dw for the Futian mangrove wetlands and Shenzhen Bay, China, respectively (Deng *et al.*, 2015); and 0.3–24.4 μg kg^{-1} dw in the Mediterranean and the Atlantic (Anastasiou *et al.*, 2016).

When we compared the concentrations of TBT in water and sediment samples to those of TPT, we found that the water samples contained higher concentrations of TBT than TPT, but the sediment samples contained similar levels of TBT and TPT. This was because TPT has lower water solubility than TBT.

Distribution of OT compounds in water and sediment samples

We next examined the horizontal and vertical distributions of OTs in the water column (Figure 2). In spite of the different uses of the coastal area among the stations, there were no drastic differences in TBT concentrations in the surface water samples

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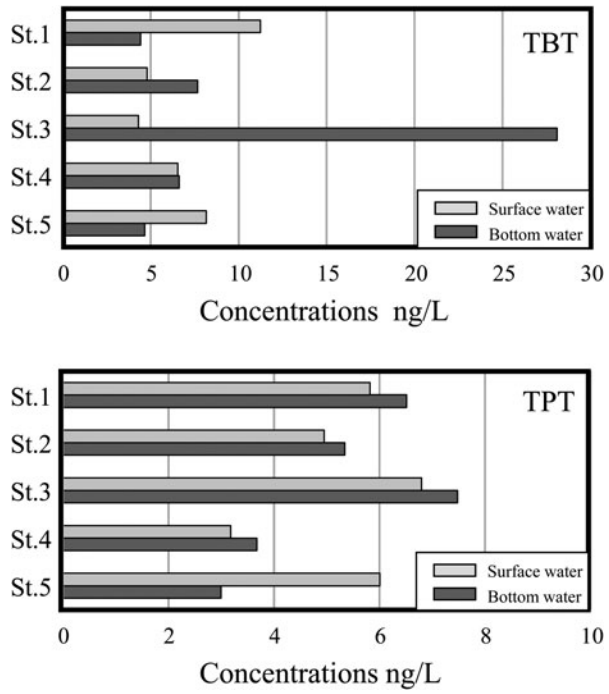

Fig. 2. Concentrations of tributyltin (TBT) and triphenyltin (TPT) in the water column.

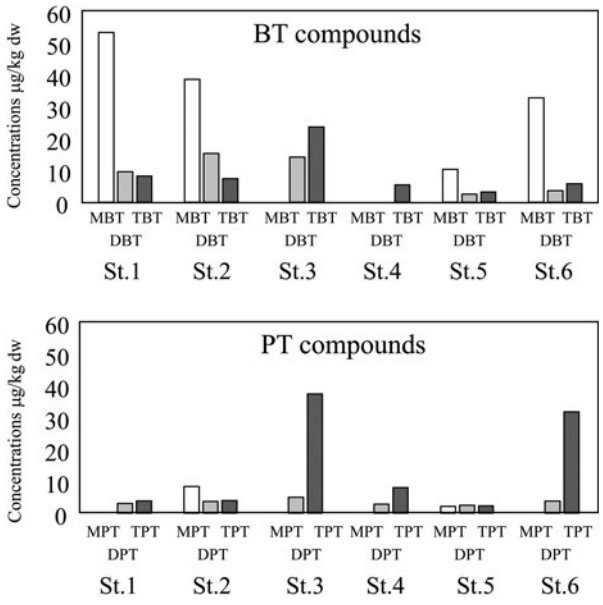

Fig. 3. Horizontal distributions of butyltin (BT) and phenyltin (PT) compounds in sediment.

from Stations 1–5 except at Station 3, which shows that TBT was not used as an antifouling paint. In the case of Station 3, the TBT concentrations in the bottom water were the highest among the sampling sites (Figure 2), and TBT accounted for a larger percentage of the total BTs in the bottom water samples than in the surface water samples at this station (Figure 4). The concentration of TBT in sediment was also highest at Station 3, and TBT was the dominant species among BTs at Station 3 (Figure 3). Adult yellowtail and red sea bream were being farmed at Station 3, and TBT was used as an antifouling paint at the aquafarm. In addition, the water exchange at this site was poor. Therefore, TBT that had been eluted from the aquaculture nets and equipment into the water column was accumulated in the sediment. Because TBT persists in sediment for a long time (Maguire & Tkacz, 1985; de Mora *et al.*, 1989; Dowson *et al.*, 1993; Harino *et al.*, 1998), the sediment at Station 3 was still heavily contaminated by TBT. The higher TBT concentrations in the bottom water were considered to be due to the elution of TBT from the sediment.

Although no drastic differences in the concentrations of TPT were observed in the surface water among the sampling sites, the concentrations in sediment from Stations 3 and 6 were high (Figures 2 and 3). It was suggested that TPT was also used in aquaculture facilities as an antifouling paint, because Stations 3 and 6 were both located in areas where adult yellowtail and red sea bream were being farmed. In spite of the higher concentrations of TPT in sediment at Stations 3 and 6, no differences in the TPT concentrations in the surface and bottom water samples were observed among stations (Figure 2). Because TPT has hydrophobic characteristics, the amount of TPT that was eluted from sediment was low in comparison with the amount of TBT. This is why the TPT concentrations in the sediment were not reflected in the water sample.

Concentrations of OT compounds in biological samples

The concentrations of BTs and PTs in biological samples are shown in Figure 5. The concentrations of TBT in gastropods and oysters ranged from 5.1–106 µg kg^{-1} ww and 2.3–117 µg kg^{-1} ww, respectively. Anastasiou *et al.* (2016) reported the TBT concentrations in gastropods from the Mediterranean and the Atlantic: TBT was detected in the range of 9.1–189.7 µg kg^{-1} in gastropods (*Hexaplex trunculus*) in the ports of Cagliari, (Sardinia), El Kantaoui (Tunisia) and Olhão (Portugal), and the TBT concentrations in gastropods (*H. trunculus* and *Nassarius nitidus*) collected from the port and Ria Formosa Lagoon in Portugal were in the range of 5.8–15.4 µg kg^{-1}. Deng *et al.* (2015) reported that TBTs were detected in the range of <0.269–2.3 µg kg^{-1} in gastropods (*Onchidium verruculatum* and *Ellobium chinense*). TBT was detected in the range of <5–50 µg kg^{-1} in gastropods collected along the coastal areas of Panama (Batista-Andrade *et al.*, 2018). The concentrations of TBT in biological samples from Tanabe Bay were higher than the reported values. Deng *et al.* (2015) reported that the TBT concentration in bivalves (*Geloina coaxans*) from the Futian mangrove wetlands in China was 1.9 µg kg^{-1}. The TBT concentration in blue mussels (*Mytilus galloprovincialis*) collected along the Croatian Adriatic coast was 170 µg kg^{-1} (Furdek *et al.*, 2012). The concentrations of TBT in bivalves collected from Tanabe Bay were also higher than in other sites.

The TBT concentrations in gastropods from Station 10 were lower than those in gastropods collected from other stations in Tanabe Bay (Figure 5). This suggested that the bivalves and gastropods that inhabited Station 10 were less affected by water and sediment in comparison with those located at the other stations, because Station 10 was located in the intertidal zone.

The concentrations of TPT in gastropods and oysters collected from Tanabe Bay ranged from 5.9 −115.2 µg kg^{-1} and 3.5–104.9 µg kg^{-1}, respectively. These values were higher than those in gastropods and oysters from other areas. Indeed, Anastasiou *et al.* (2016) reported that TPT was not detected at all in the gastropods *H. trunculus* and *N. nitidus* in the port or in gastropods from Ria Formosa Lagoon in Portugal. Deng *et al.* (2015) reported that the TPT concentration ranged from 10–11.5 µg kg^{-1} in gastropods and was 24.9 µg kg^{-1} in bivalves in the Futian mangrove wetlands in China, while Furdek *et al.* (2012) reported that the TBT concentration ranged from 64–124 µg kg^{-1} in *M. galloprovincialis* collected along the Croatian Adriatic coast.

The concentrations of TPT in gastropods and oysters were high at Station 9 in Tanabe Bay, reflecting the concentrations of TPT in the sediment.

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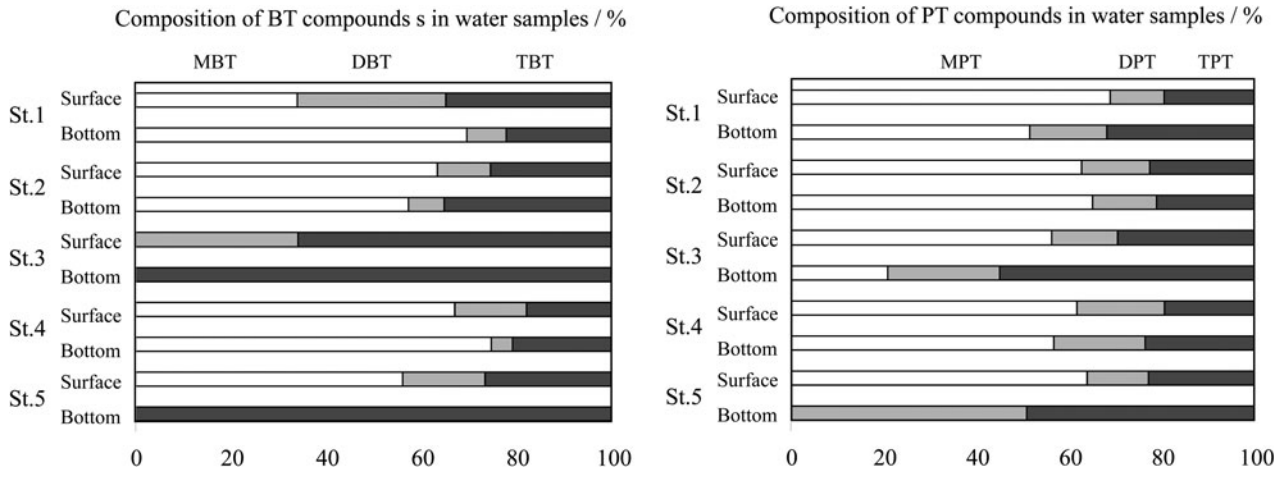

Fig. 4. Compositions of BT and PT compounds in the water column.

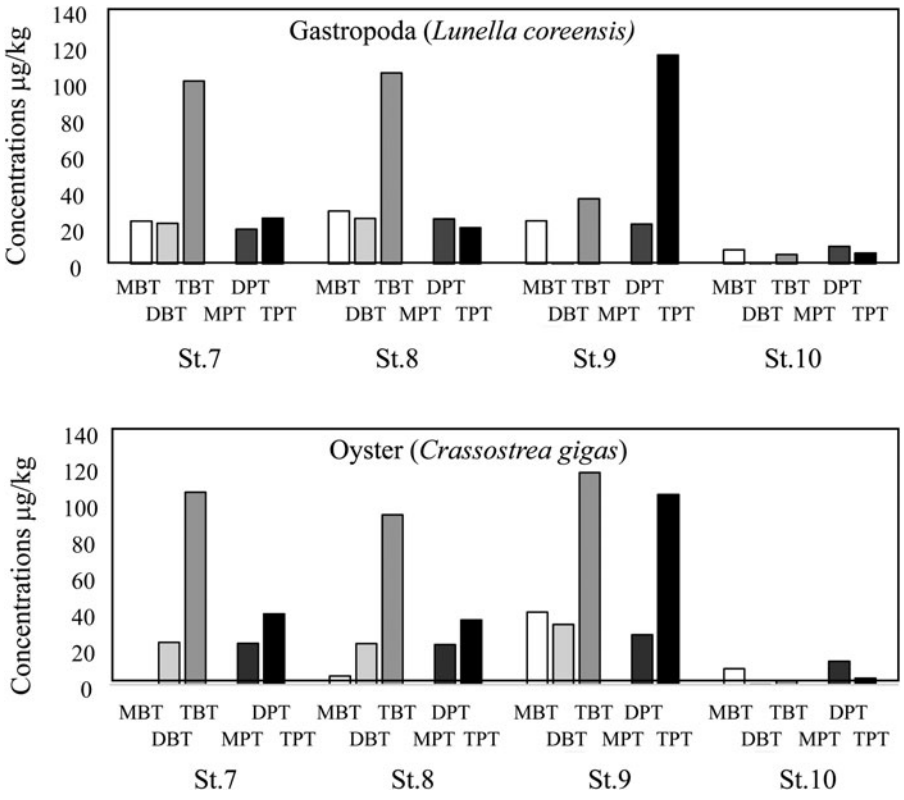

Fig. 5. Concentrations BT and PT compounds in gastropods and oysters.

The TBT concentrations in the four species of fish taken from Tanabe Bay ranged from 1.1–4.9 µg kg^{-1} and were all at similar levels (Figure 6). These values were lower than previously reported concentrations – e.g. TBT concentrations of <0.2–904.4 µg kg^{-1} were detected in commercial fishes collected from the west coast of India (Jadhav *et al.*, 2011). Moreover, the concentrations of TBT in the four species of fish in Tanabe Bay were lower than those in gastropods and oysters. The likely reason for this is that the habitats of the fish were wider than those of oysters and gastropods. MBT, which is a product of the degradation of TBT, was the dominant species among BTs in these fish.

At <0.2–3.9 µg kg^{-1}, the TPT concentrations in fish from Tanabe Bay were also lower than those in gastropods and oysters. This is attributed to the wide range of fish habitats.

Because the oysters and fish sampled in the present study are important food sources in the Japanese diet, the presence of TBT in oysters and fish may pose a health risk in Japan. Penninks (1993) derived a tolerable daily intake (TDI) of TBT of 0.25 mg kg^{-1} body weight. This TDI value, along with the average body weight (50 kg) of Japanese and the average daily seafood consumption (77.8 g) in the Japanese population (Fisheries Agency, 2018), can be used to determine the tolerable average residue levels (TARLs) for seafood based on the following formula:

TARL = (TDI × 50 kg body weight)/(average daily seafood consumption)

In this manner, the TARL for seafood in Japan is estimated to be 161 µg kg^{-1}, and the concentrations detected in oysters and fish from Tanabe Bay were well below this value.

Distribution of alternative biocides

Many antifouling biocides were used after the ban of OTs by the IMO. Pyrithiones and organoborons are commonly used as alternatives. Unfortunately, the measurement of these compounds is

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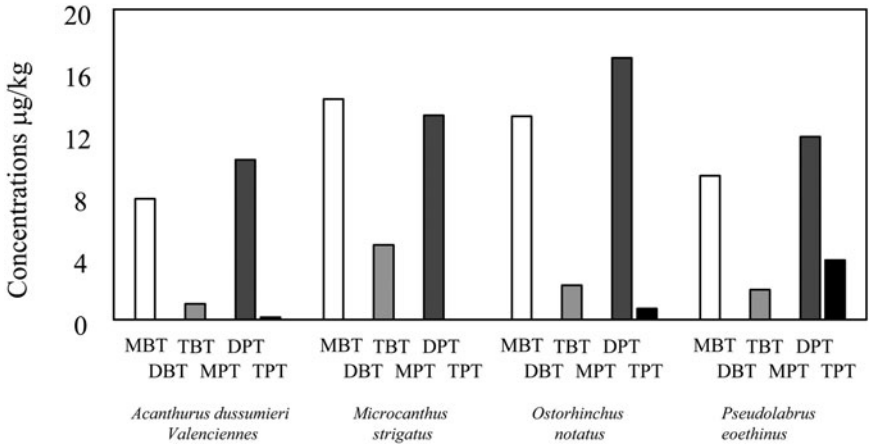

Fig. 6. Concentrations of BT and PT compounds in fish.

Table 4. Concentrations (ng l^{-1}) of alternative biocides in surface and bottom water samples from Tanabe Bay

Station	Sample	Sea-Nine 211	Diuron	Dichrofluanid	Chlorothalonil	Irgarol 1051	M1
Station 1	Surface water	<0.1	16	44	20	<0.1	11
	Bottom water	13	<0.1	24	15	<0.1	6
Station 2	Surface water	<0.1	<0.1	23	14	<0.1	5
	Bottom water	31	<0.1	40	21	<0.1	14
Station 3	Surface water	<0.1	<0.1	<0.1	26	<0.1	21
	Bottom water	<0.1	27	79	37	<0.1	25
Station 4	Surface water	17	16	13	8	2	8
	Bottom water	<0.1	<0.1	13	8	<0.1	4
Station 5	Surface water	<0.1	<0.1	17	8	<0.1	6
	Bottom water	<0.1	<0.1	8	8	2	2

The numbers in parentheses show the relative standard deviation.

prohibitively difficult, so we measured the concentrations of alternative biocides other than pyrithiones and organoborons in Tanabe Bay. The concentrations of diuron, chlorothalonil, dichlofluanid, irgarol 1051 and Sea-Nine 211 in water samples from Tanabe Bay were in the ranges of <0.1–27 ng l^{-1}, 8–37 ng l^{-1}, <0.1–44 ng l^{-1}, <0.1–2 ng l^{-1} and <0.01–31 ng l^{-1}, respectively (Table 4). There have been many reports of the concentrations of diuron and irgarol 1051 in water samples (Harino, 2016). Diuron and irgarol 1051 have been detected in the ranges of <0.7–1540 ng l^{-1} and <0.1–2430 ng l^{-1}, respectively, worldwide. There have been fewer reports concerning the concentrations of chlorothalonil, dichlofluanid and Sea-Nine 211, but these compounds were found to range from <1–1380 ng l^{-1}, <1–55 ng l^{-1} and <1–3700 ng l^{-1}, respectively, in England, Spain, Greece and Japan (Harino, 2016). Collectively, these data demonstrate that the levels of alternative biocides in Tanabe Bay were lower than those in the other countries.

The horizontal distributions of chlorothalonil and dichlofluanid in the surface water of Tanabe Bay were assessed because these compounds were the most frequently detected among the alternative biocides. No clear trend in the horizontal distribution of these compounds in water samples was observed.

The concentrations of chlorothalonil and dichlofluanid in surface water were compared with those in bottom water. No differences in either compound were observed between the two locations. Therefore, it is considered that these compounds were introduced to coastal areas both from ship hulls and from the river, because these compounds are used as both pesticides and antifouling biocides. In spite of the lower detection frequency of irgarol 1051 in water samples, M1, which is a product of the degradation of irgarol 1051, was detected in the range of 2–25 ng l^{-1} in water samples. That the concentration of M1 in seawater was higher than that of irgarol 1051 was attributed to the degradation of irgarol 1051, since it has been reported that irgarol 1051 is easily degraded to M1 in water samples (Liu *et al.*, 1997; Okamura *et al.*, 1999).

Among the alternative biocides, chlorothalonil and irgarol 1051 were detected in sediment (Table 5). The concentrations of chlorothalonil and irgarol 1051 ranged from 0.1–8.2 µg kg^{-1} dry and 8.2–9.3 µg kg^{-1} dw, respectively. Chlorothalonil and irgarol 1051 were detected in the range of <0.1–460.5 µg kg^{-1} dry and <0.1–816 µg kg^{-1} dw, respectively, in England, Japan and Malaysia (Harino, 2016). The concentrations of these alternative biocides in Tanabe Bay were lower than those in the other countries. Irgarol 1051 was detected in sediment from all of the sampling sites, suggesting that irgarol 1051 absorbed on suspended solids (SS) is stable and that SS containing irgarol 1051 was dropped into the sediment.

The concentrations of dichlofluanid, chlorotharonil and irgarol 1051 ranged from <0.1–358 µg kg^{-1}, 53–280 µg kg^{-1} and <0.1–48 µg kg^{-1} in gastropods, respectively (Figure 7). The concentrations of these compounds in gastropods were not compared with those at other sites, because there have been no reports on the concentrations of alternative biocides in gastropods. The concentrations of dichlofluanid, chlorotharonil and irgarol 1051 in oysters ranged from <0.1–117 µg kg^{-1}, <0.1–198 µg kg^{-1} and

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Table 5. The concentrations (μg kg^{-1} dw) of alternative biocides in sediment samples from Tanabe Bay

Station	Sea-Nine 211	Diuron	Dichlofluanid	Chlorothalonil	Irgarol 1051	M1
Station 1	<0.1	<0.1	<0.1	6.3	8.3	<0.1
Station 2	<0.1	<0.1	<0.1	7.4	9.1	<0.1
Station 3	<0.1	<0.1	<0.1	<0.1	8.2	<0.1
Station 4	<0.1	<0.1	<0.1	7.7	8.7	<0.1
Station 5	<0.1	<0.1	<0.1	8.0	8.8	<0.1
Station 6	<0.1	<0.1	<0.1	8.2	9.3	<0.1

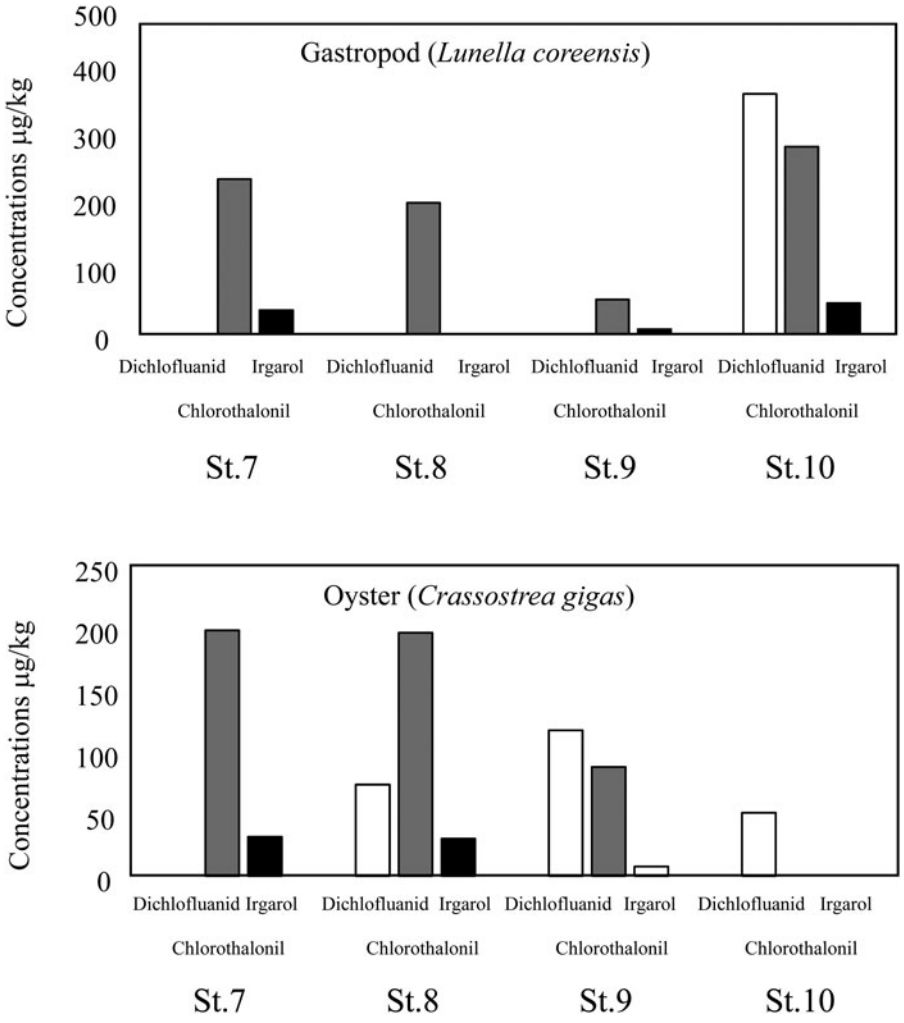

Fig. 7. Concentrations of alternative biocides in gastropods and oysters.

<0.1–32 μg kg^{-1}, respectively (Figure 7). Harino *et al.* (2010) reported that irgarol 1051 was detected in the range of 1.1–4.7 μg kg^{-1} in oysters and mussels in the coastal areas of Awaji Island, Japan (Harino *et al.*, 2010). The concentrations of irgarol 1051 in Tanabe Bay were higher than those near Awaji Island. There have been no reports concerning the concentrations of dichlofluanid and chlorotharonil in oysters. Therefore, the concentrations of dichlofluanid and chlorotharonil in Tanbabe Bay could not be compared with the other sites. Although the concentrations of irgarol 1051 were low, dichlofluanid, chlorotharonil and irgarol 1051 were detected in gastropods and oysters. The detection of these compounds in oysters and gastropods was considered to be due to the accumulation of these compounds in water samples, because oysters and gastropods are filter feeders.

Dichlofluanid, chlorotharonil and irgarol 1051 were detected in fish in the ranges of 325–339 μg kg^{-1}, 268–291 μg kg^{-1} and 43–49 μg kg^{-1}, respectively (Figure 8). This is the first finding that antifouling biocides have been detected in fish. The concentrations of these compounds in fish were similar to those in gastropods and oysters in Tanabe Bay.

The additional daily intake (ADI) levels of dichlofluanid and chlorotharonil were 0.018 mg kg day^{-1} and 0.3 mg kg day^{-1}, respectively. The TARLs calculated from the ADIs were 12 μg kg^{-1} wet weight and 193 μg kg^{-1} wet weight, respectively. The concentrations of chlorothalonil and dichlofluanid in biological samples were over the TARLs. The TARL of irgarol 1051could not be calculated, because the ADI of irgarol 1051 was not reported. The mixture toxicity of alternative compounds is a

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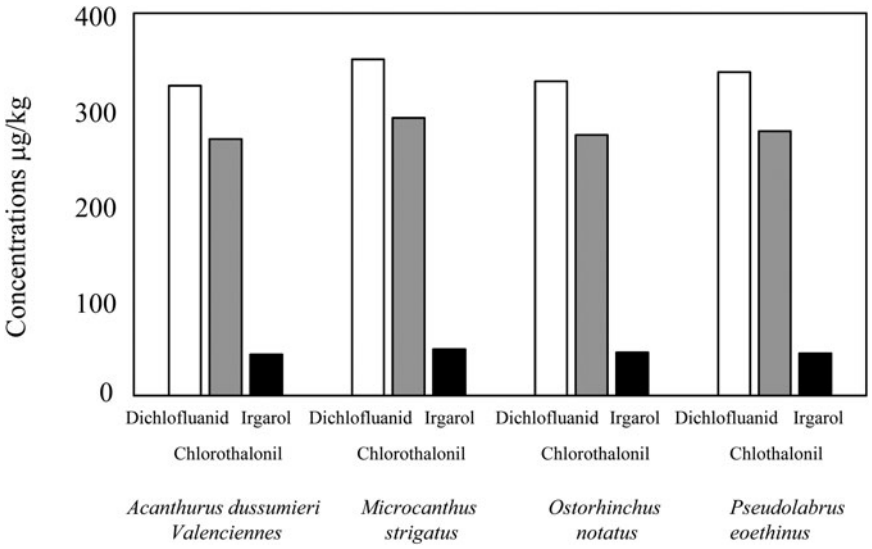

Fig. 8. Concentrations of alternative biocides in fish.

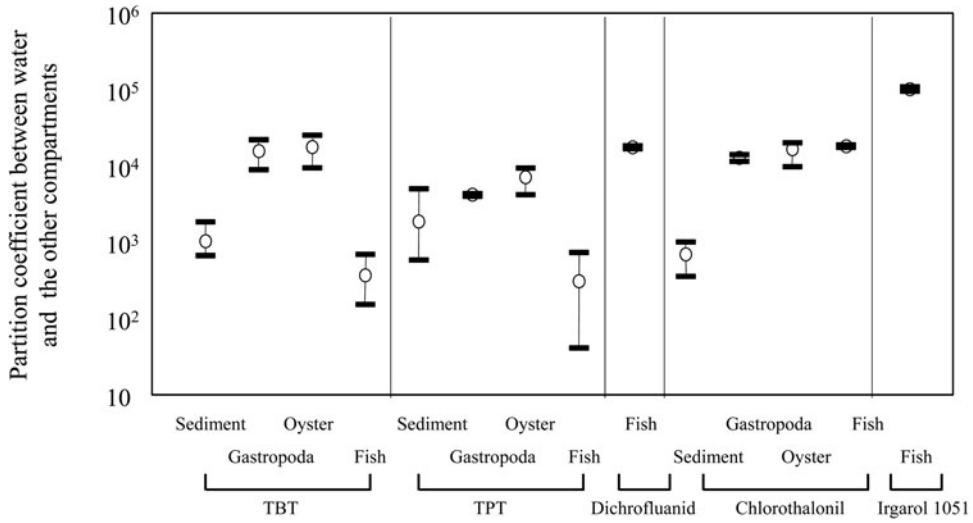

Fig. 9. Partition coefficients between water samples and sediment and biological samples.

concern, because some alternative biocides were accumulated in fish. There have been no reports of mixture toxicity to humans. However, there have been a few reports on mixture toxicities for aquatic organisms. Koutsaftis & Aoyama (2007) reported the changes of 24-h LC_{50} due to binary mixtures for brine shrimp, *Artemia salina*. The mixtures of chlorothalonil and copper pyrithiones had an antagonistic effect. Fernández-Alba *et al.* (2002) reported the mixture toxicities of alternative biocides for *Vibrio fischeri*, *Selenastrum capricornotum* and *Daphnia manga*, showing that a binary mixture of irgarol 1051 and diuron gave a synergistic effect. Given the effects of mixture toxicity on aquatic organisms, it is necessary to study the mixture effects of these compounds on humans.

Partitions of antifouling biocides in various compartments

The partition coefficients for various compartments were calculated by dividing the concentrations in sediment and biological samples by the concentrations in water samples (Figure 9). The partition coefficients of TBT and TPT could be arranged in the following order from highest to lowest: gastropods = oyster > sediment > fish. The partition coefficients of TBT and TPT in sediment were low, because the origin of these compounds in water samples was their elution from sediment.

On the other hand, the partition coefficients of alternative biocides could be arranged in the following order from highest to lowest: fish > gastropoda = oyster > sediment. Chlorotharonil and dichlofluanid are used as pesticides as well as in antifouling paints, and the coastal area of Tanabe Bay was widely contaminated with these compounds. Therefore, the partition coefficients of the biological samples were considered to be higher than those in the sediment, because the biological samples used in this study show higher absorption of these compounds from water in comparison to sediment. The partition coefficients of chlorothalonil, dichrofluanid, and irgarol 1051 were similar to those of OTs.

In general, there have been few studies on the concentrations of alternative biocides in biological samples; further studies on the accumulation of alternative biocides in biological samples are warranted.

Conclusion

Despite the fact that it was banned in 1990, TBT was detected in our water and sediment samples from Tanabe Bay. TPT was also detected in these samples. It was suggested that the presence of

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higher TBT concentrations in bottom water was due to the elution of TBT in sediment. Moreover, although TBT and TPT were detected in biological samples, their concentrations were lower than the TARLs of those compounds. Among alternative biocides, dichlofluanid, chlorotharonil and irgarol 1051 were detected in water, sediment and biological samples, and the concentrations of dichlofluanid and chlorotharonil were near the TARL values.

Recently, in addition to the alternative biocides that were researched in this study, various chemical substances such as zinc pyrithiones and organoborons have been used as antifouling biocides. It will be necessary to monitor all the antifouling biocides that contain OT compounds for the purpose of protecting aquatic organisms.

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