Current Status of Antifouling Biocides Contamination in the Seto Inland Sea, Japan

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Response to Reviewers:	I would also like to thank you for your detail comments. By the way, I used the word "significantly" to indicate that there was a significant difference in statistical analysis, but I apologize for using the wrong English. As I wrote in my comment to the reviewers, environmental analysis, especially analysis of chemical analysis such as antifouling biocides, requires a lot of time and amount of sample. Therefore, it is difficult to obtain enough data for statistical analysis. So, we conducted a recovery test as described in the section of analysis method. The

accuracy of the measurement data is predicted from these results. I would be happy if you could read this manuscript with these considerations in mind. I will write below these corrections to the comments that you have checked in detail. As the corrections are written in red, please check them. By the way, the line numbers when indicating corrections are slightly different from the line numbers in PDF. I couldn't use in line numbers in PDF, because the line numbers would be added before the submission was complete.

Page 1 (abstract), line 21: Correct the spelling of “biocides”.
>I followed the editor's comments and made the corrections. The revised parts are written in red. Please see at page1, line8.

Page 2, line 11: Change “species” to “homologues”.
>I followed the editor's comments and made the corrections. The revised parts are written in red. Please see at page2, line4

Page 4, line 40: Change to “Stn 02, which is located…”
>I followed the editor's comments and made the corrections. The revised parts are written in red. Please see at page

Page 4, line 53: Change “carried out” to “located”.
>I followed the editor's comments and made the corrections. Please see at page4, line16 and 21.

Page 7, line 48: Change to, “…calculated by multiplying the signal to noise ratio by three”.
>I followed the editor's comments and made the corrections. Please see at page7, line4 and page9, line2.

Page 9, line 52: Change to, “The range of concentrations of TBT…”.
>I followed the editor's comments and made the corrections. Please see at page9, line2

Page 10, line 56: Rather than saying that there were no “notable” differences in concentrations, I assume you can say that there were no statistically significant differences in concentrations, based on your t-tests?
>No statistical analysis has been performed for this. So I changed the expression to " TBT concentrations were similar values among all sampling sites in this study." Please see at page11, line4.

Page 11, lines 43-45: Please change to, “In general, there was a trend for concentrations of TBT in water collected at the bottom to be higher than TBT concentrations collected from surface water, but these trends were not analyzed statistically because of the small number of data points”.
>I followed the editor's comments and made the corrections. Please see at page11,line11

Pages 14 to 22: Report on the statistical analysis of alternative biocides in water and sediments between the two sampling locations. Also, in comparing water samples, you report that concentrations of alternative biocides in bottom water were higher than concentrations in surface water. However, without statistical analysis, you can only report these as trends, and you must state that there was no statistical analysis to verify these trends.

>It was clarified whether the results were compared through statistical analysis. It is written in red, so please check it.

Table 3: Make sure that all decimal notations are with a period and not a comma (e.g., 2.7, not 2,7).

>Thank you for your pointing out. I have corrected the numbers in the table.

Page 10 (top): Report on the statistical analysis of differences in concentrations of TBT

	and total BTs in water collected at the two sampling sites. Page 12 (top): Report on your statistical analysis of differences in the concentrations of TBT and total BTs in sediments collected from the two sites. Page 13: Report on statistical analysis of TPTs and total PTs. > I have written it so that you can distinguish between comparisons that have been statistically analyzed and comparisons that have not been analyzed. Please check the parts written in red.

Current Status of Antifouling Biocides Contamination in the Seto Inland Sea, Japan

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Running title : Distribution of antifouling biocides in Seto Inland Sea, Japan

Headings:

Organotin compounds and some alternative biocides were detected in water and sediment samples from Seto Inland Sea, Japan
The concentrations of TBT was the values which may affect on aquatic animals
The concentrations of alternative biocides were lower than those which effect on aquatic organisms.

Abstract

A monitoring survey of antifouling biocides was conducted in the Harima Nada Sea and Osaka Bay of the Seto Inland Sea, Japan to assess contamination by organotin (OT) compounds and alternative biocides. The concentrations of tributyltin (TBT) compounds in surface water ranged from 1.0 to 2.8 ng/L, and the detected TBT concentrations in the bottom water layer were higher than those in the surface water, The concentrations of TBT compounds in sediment samples ranged from 2.0 to 28 ng/g dry weight (dw) respectively.

The concentrations of alternative biocides in the water and sediment were lower than those before the banning of TBT by the International Maritime Organization (IMO). Although triphenyltin (TPT) compounds were not detected in water samples, TPT compounds were detected in the range of <0.1–2,700 ng/g dw in sediment samples. Their concentrations in the water samples were as follows: diuron, <1–53 ng/L; Sea-Nine 211, <1–1.8 ng/L; Irgarol 1051, <1–4.0 ng/L; dichlofluanid, <1–343 ng/L; and chlorothalonil, <1–1 ng/L, and the ranges of these alternative compounds in sediment samples were diuron, 32–488 ng/g dw; Sea-Nine 211, 47–591 ng/g dw; Irgarol, 33–128 ng/g dw; dichlofluanid, 67–8,038 ng/g dw; and chlorothalonil, 31–2,975 ng/g dw. Thus, the OTs and alternative biocides have still been detected in water and sediment samples from closed sea areas.

Introduction

Organotin (OT) compounds have been used as active biocides in antifouling paints since the early 1960s. Organotin compounds released into the water column from coatings applied to ship hulls have caused deleterious effects such as endocrine disruption in non-target marine organisms (Laughlin and Linden 1985; Bryan and Gibbs 1991; Ohji et al. 2002, 2003), and environmental research has indicated OT contamination in marine environments worldwide (Clark et al. 1988).

In the 1980s, the use of tributyltin (TBT) was regulated in some developed countries including England, France, and the USA. In Japan, bis(tributyltin)oxide (TBTO) was banned in 1990 by a domestic law, but the use of seven TPT homologues and thirteen TBT homologues other than TBTO remained allowed if the ship owner has a certificate permitting the use of OTs. Despite the regulation of OTs in developed countries, OT compounds are still detected at high concentrations in water, sediment, and biota from harbors, marinas, and estuaries, particularly where boat activity is high and water movement is restricted (Harino et al. 1998, 2000). In 2001, the International Maritime Organization (IMO) adopted the International Convention on the Control of Harmful Antifouling Systems (AFS Convention), which prohibits the use of OTs as active ingredients in antifouling systems for ships.

Ho et al. (2016) reported that (*i*) the concentrations of OTs in the tissue of rock shells (*Reishia clavigera*) collected near Hong Kong did not decline between 1990 and 2015, and (*ii*) the concentrations of TPT were especially high. They also demonstrated that imposex induced by TBT and TPT was present in 100% of the individual *R. clavigera* examined. Egardt et al. (2017) detected BTs (butyltins), irgarol 1051 (2-methylthio-4-tert -butylamino-6-cyclopropylamino-s-triazine) and diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) in sediment from a national park in Sweden, despite the ban on TBT within the European Union (EU). In an investigation reported by Concha-Grana et al. (2021), the butyltin degradation index (BDI) values were <1 in some sediment samples from semiconfined areas of the Spanish coast, suggesting a new input of TBT and thus indicating that OT contamination has continued despite the global regulation of OT compounds.

Following the international restrictions on the use of OT-based antifoulants, some chemical substances have been used as alternative biocides. Representative alternative biocides include Sea-Nine 211 (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one), diuron, Irgarol 1051, chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), dichlofluanid (N,N-dimethyl-N'-phenyl-N'-(dichlorofluoromethylthio) sulfamide), copper pyrithione (bis(2-sulfidepyridine-1-olato) copper(II)), zinc pyrithione (zinc 2-pyridinethiol-1-oxide), and pyridine triphenyl borane. Studies based on marine environmental surveys have indicated that the detection frequencies and concentrations of copper pyrithione, zinc pyrithione, and pyridine triphenyl boran in water and sediment samples were low, whereas the concentrations of Sea-Nine 211, diuron, Irgarol 1051, chlorothalonil, and dichlofluanid were high (Harino et al. 2009; Mochida et al. 2012; Egardt et al. 2017; Harino and Yamato 2021). Diuron and Irgarol 1051 in particular have been detected in marine environments in the EU even though their use is banned there.

It is therefore necessary to evaluate the data obtained by monitoring surveys to determine whether the use of OT compounds has truly been eliminated at present and whether alternative biocides have had an impact on marine environments.

Closed sea areas and their coasts have long been used as fishing grounds because they are blessed with a calm natural environment, and they have also been developed as bases for various activities such as industry and foreign trade. Pollutants tend to accumulate in closed sea areas, and it can be difficult to maintain the water quality in these areas because the water exchange is poorer than that in the open sea. The Seto Inland Sea is one of the ~20 closed sea areas in the world. It is the body of water separating three (Honshu, Shikoku, and Kyushu) of the four main islands of Japan. According to a water-quality monitoring survey of the Seto Inland Sea by Japan's Ministry of the Environment (Ministry of the Environment 2021), the water quality of the Seto Inland Sea has improved in the last 20 years with respect to the chemical oxygen demand (COD), total nitrogen, and total phosphorous.

However, it has also been reported that various artificial chemical substances were detected in water, sediment, and biological samples in this aquatic area (Asaoka et al. 2019; Goto et al. 2017). The Seto Inland Sea is thus highly suitable for the assessment of antifouling biocide contamination.

In the present study, the concentrations of antifouling biocides were surveyed in water and sediment samples from the Seto Inland Sea. Based on these results, we discuss both whether this sea's OT contamination has continued and the current status of alternative biocide contaminations.

Materials and Methods

Sampling

In 1973, the Seto Inland Sea was divided into twelve sea areas by Japan's Act on Special Measures Concerning the Conservation of the Environment of the Seto Inland Sea. The Harima Nada Sea and Osaka Bay, which were sampled in the present study, are located in the eastern part of the Seto Inland Sea. The basin areas of the Harima Nada Sea and Osaka Bay are 3,426 km^2 and 1,447 km^2, and the mean water depths are 25.9 m and 30.4 m, respectively. As shown in Figure 1, the water direction in Osaka Bay is clockwise and the water direction in the Harima Nada Sea flows westward from Osaka Bay. Station (Stn) O1 is located at the mouth of the Yodo River, which has the 7th largest river basin area in Japan; Stn O2 which is located at the mouth of the Yodo River, is affected by the Yodo River.

According to the Ministry of the Environment (2021), water samples from the Harima Nada Sea and Osaka Bay showed the following respective values: COD, 4.8–7.5 mg/L and 1.5–6.7 mg/L; total nitrogen, 0.09–0.56 mg/L and 0.17–1.3 mg/L; and total phosphorous, 0.015–0.034 mg/L and 0.014–0.130 mg/L. Although the Harima Nada Sea and Osaka Bay are relatively clean sea areas, various industries are located around the sampling sites as shown in Table 1.

In the present study, surface water samples were collected in September 2017 from all fifteen sampling sites, and bottom water samples were collected from Stns. H1, H3, H6, and H9 for the Harima Nada Sea and Stn. O1 for Osaka Bay in the Seto Inland Sea with the use of a Van Dorn water sampler (Fig. 1, Table 1). Sediment samples were collected by a Smith-McIntyre grab sampler in November 2018. The water samples were stored at 3°C after being collected and were analyzed within 10 days. Sediment samples were stored at −20°C until analysis.

Analytical Measurement

Organotin compounds

For the measurement of OT compounds, 1 L of water sample was placed in a separation funnel, and 50 μL of a mixed acetone solution containing 1 μg/mL each of monobutyltin trichloride (MBTCl$_3$)-d$_9$ (Kishida Chemicals, Osaka, Japan), dibutyltin dichloride (DBTCl$_2$)-d$_{18}$ (Tokyo Chemical Industry, Japan), tributyltin monochloride (TBTCl)-d$_{27}$ (Tokyo Chemical Industry, Japan), monophenyltin trichloride (MPTCl$_3$)-d$_5$ (Strem Chemicals, Newburyport, MA, USA), diphenyltin dichloride (DPTCl$_2$)-d$_{10}$ (Aldrich Japan) and triphenyltin monochloride (TPTCl)-d$_{15}$ (Tokyo Chemical Industry, Japan) was added to the funnel as a surrogate standard. Surrogate compounds were used to correct for the losses in pretreatment and the variability of values by gas chromatography/mass spectrometry (GC/MS). Five milliliters of acetic acid-sodium acetate buffer (pH 5.0) and 1 mL of 5% sodium tetraethyl borate (NaBEt$_4$) were also 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 up to 1 mL by a rotary evaporator, the final solution was concentrated up to 0.5 mL in a nitrogen atmosphere. The analytes were determined by GC/MS.

The method used for the determination of OTs in sediment samples was based on that of

Midorikawa et al. (2004), with some modification. A 2-g aliquot of the sediment was added to a centrifuge tube, and then 100 µL of mixed acetone solution containing 1 mg /L each of MBTCl-d_9, DBTCl-d_{18}, TBTCl-d_{27}, MPTCl-d_5, DPTCl-d_{10}, and TPTCl-d_{15} was added to the centrifuge tube as a surrogate standard. The mixture was shaken with 15 mL of 1 M HCl-methanol/ethyl acetate (1/1) for 10 min. After centrifugation for 10 min, the residue was again extracted with 10 mL of 1 M HCl-methanol/ethyl acetate (1/1). After centrifugation, the combined supernatants and 30 mL of saturated NaCl solution were transferred to a separatory funnel. The analytes were extracted twice with 15 mL of ethyl acetate/hexane (3/2) solution, and the organic layer was combined.

Fifty milliliters of hexane were 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 concentrated up to 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 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 a 1 M KOH–ethanol solution by shaking for 1 hr. Forty milliliters of distilled water and 20 mL of hexane 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 20 mL of hexane. The combined organic layers were dried with anhydrous Na_2SO_4.

After being concentrated up to 1 mL, the solution was cleaned by using a Florisil Sep-Pak column (Waters, Milford, MA). The analytes were eluted with 10 mL of 5% diethyl ether/hexane. All of the eluting solvent was collected in a bottom flask. After being concentrated up to 1 mL by a rotary evaporator, the final solution was concentrated up to 0.5 mL in a nitrogen atmosphere. The analytes were determined by GC/MS.

The standard reagents of monobutyltin (MBT), dibutyltin (DBT), TBT, monophenyltin (MPT), diphenyltin (DPT), and TPT used for the calibration curve were purchased from Hayashi Pure Chemicals (Osaka, Japan). One mg/L calibration solution containing each OT compound was adjusted. Their diluted solutions were used for the calibration curve. The range of concentrations of TBT in calibration curve were 0.001 – 1 mg/L. The detection limit were calculated by multiplying the signal to noise ratio by three

A Hewlett-Packard 7890A series gas chromatograph equipped with a mass spectrometer (5973 N) was used for the analysis of the OTs. The separation was carried out in a capillary column coated with 5% phenyl methyl silicone (30 m length × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA). The column temperature was held at 60°C for the first 2 min, then increased to 130°C at 20°C/min, to 210°C at 10°C/min, to 260°C at 5°C/min, and to 300°C at 10°C/min. Finally, the column temperature was held at 300°C for 2 min. The interface temperature, ion source temperature, and ion energy were 280°C, 230°C, and 70eV, respectively. Selected ion monitoring was performed under this program. The monitoring ions and the qualifier ions are shown in Table 2. One microliter of the sample was injected using splitless injection. The concentrations of OTs in this study are expressed as Sn^{4+}.

When 1 μg of OTs was spiked to a 1-L water sample, the recovery rates and relative standard deviations (RSDs) of the OTs were 73%–100% and 3.0%–7.5%, respectively. When 1 μg of OTs was spiked to 2 g of sediment samples, the recovery rates of the OTs ranged from 95% to 117% and their RSDs ranged from 6.1% to 11%. The detection limits were calculated from a signal-to-noise ratio of 3. The detection limit of each OT in the water samples was 0.1 ng/L. The detection limit of each OT in the sediment samples was 0.1 ng/g dry weight (dw).

Alternative biocides

One liter of water sample 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 sodium sulfate (Na_2SO_4), the organic layer was concentrated by a rotary evaporator up to 1 mL. Fifty µL of hexane solution containing 0.5 mg/L of atrazine-d_5 (Hayashi Pure Chemicals, Japan) as an internal standard was added to the organic layer, and the organic layer was concentrated up to 0.5 mL in a nitrogen atmosphere. An internal standard was used to correct the variations of values in GC/MS. The analytes were determined by GC/MS.

The method used for the determination of alternative compounds in the sediment samples was based on that of Harino et al. (2005). First, 2 g of sediment was placed together with 10 mL of acetone in a centrifuge tube, and the mixture was shaken for 10 min by a mechanical shaker. After the supernatant's removal, the analytes were re-extracted with 10 mL acetone for 10 min and the mixture was then centrifuged. The combined supernatants were concentrated by a rotary evaporator up to 5 mL. Forty-five mL of distilled water, 1 g of zinc acetate, and 0.5 g of celite were added and the solution 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 rotary evaporator up to 1 mL. One hundred µL of hexane solution containing 1 mg/L of atrazine-d_5 was added to the organic layer, and the organic layer was concentrated up to 1 mL by nitrogen atmosphere. The analytes were determined by GC/MS.

The standard reagents of alternative biocides used for the calibration curve were purchased from Riedel-de Haen/Sigma Chemicals (St. Louis, MO). One mg/L calibration solution containing each alternative biocide was adjusted. Their diluted solutions were used for the standard curve The range of concentrations of TBT in calibration curve were 0.001 – 1 mg/L. The detection limit were calculated by multiplying the signal to noise ratio by three.

A Hewlett-Packard 7890A series gas chromatograph equipped with a mass spectrometer (5973 N) was used for the analyses of the alternative biocides. The separation was carried out in a capillary column coated with 5% phenyl methyl silicone (30 m length × 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific). The column temperature was held at 60°C for the first 1 min, then increased to 200°C at 10°C/min, and to 280°C at 5°C/min. The interface temperature, ion source temperature, and ion energy were 280°C, 230°C, and 70 eV, respectively. Selected ion monitoring was performed under this program. The monitoring ions and the qualifier ions of the alternative biocides are shown in Table 2. One microliter of the sample was injected with splitless injection.

When 1 µg of an alternative biocide was spiked to a 1-L water sample, the recovery rates and RSDs of the alternative biocides ranged from 65% to 97% and from 8.5% to 13%, respectively. When 1 µg of an alternative biocide was spiked to 2 g of a sediment sample, the recovery rates of the alternative biocides were 66%–123% and their RSDs were 7.2%–14%. The detection limits were calculated from a signal-to-noise ratio of 3. The detection limits of each alternative compound in the water and sediment samples were 1 ng/L and 0.5 ng/g dw, respectively.

Statistical analyses

Differences in the concentrations of antifouling biocides between the Harima Nada Sea and Osaka Bay were analyzed using Student's t-test (one-sided test $p<0.05$).

Results and Discussion

Organotin compounds

Seawater samples

The concentrations of TBT in the surface water samples from Harima Nada Sea and Osaka Bay were 1.0–2.8 ng/L and 1.0–2.2 ng/L, respectively and the concentrations of ΣBTs (i.e., the sum of the MBT, DBT, and TBT concentrations) ranged from 1.6 to 10 ng/L and 1.6 to 7.8 ng/L, respectively (Table 3). Several papers on TBT concentrations in water samples were published (Table 4). After the adoption of the AFS convention, the reported concentrations of TBT and ΣBTs worldwide were <0.35–393.35 ng/L and <1.13–487.27 ng/L, respectively. We observed that the concentrations of TBT in the water samples from the Harima Nada Sea and Osaka Bay were lower than these reported values. The concentration of TBT that caused chronic toxicity in larvae of rainbow trout (*Oncorhynchus mykiss)* and inland silverside *(Menidia beryllina*) fish ranged from 40 to 210 ng/L (Hall et al. 1988; de Vries et al. 1991). The concentrations of TBT that the present study detected in the Harima Nada Sea and Osaka Bay were not at a level that causes chronic toxicity in fish; however, it has been reported that a 3- to 17-ng/L concentration of TBT caused chronic toxicity in oysters (*Crassostrea gigas*) and bivalves (*Mytilus edulis*) (Lawler and Aldrich 1987: Lapota et al. 1993). In addition, Horiguchi et al. (1995) reported that TBT at the 1 ng/L level caused imposex in gastropods. Although in our present study the concentrations of TBT in water samples from the Harima Nada Sea and Osaka Bay were low, the concentrations of TBT were above the level that caused chronic toxicity in bivalves and imposex in gastropods. We thus speculate that TBT may have an adverse effect on aquatic organisms that are highly sensitive to TBT. We compared the TBT concentrations identified in this study with those in water samples from the Port of Osaka in the coastal area of Osaka Bay before the worldwide ban on TBT, which ranged from 4 to 35 ng/L (Harino et al.1998). The TBT concentrations detected in our present investigation were thus lower than those before the ban.

Table 3 provides the data of the horizontal distribution of ΣBTs in the Harima Nada Sea and Osaka Bay. Higher concentrations of ΣBTs were observed at Stns. H5–H7 in Harima Nada Sea and Stns. O1 and O5 in Osaka Bay. In contrast to the ΣBTs, TBT concentrations were similar values among all sampling sites in this study, and the TBT levels were close to the detection limits, suggesting that TBT is not currently being used as an antifouling biocide.

Diez et al. (2002) proposed the BT degradation index (BDI) and PT (phenyltin) degradation index (PDI) to quantify the degree of input and the decomposition of BT and PT compounds, respectively by their concentrations in sediment. We used the following formulas to calculate these values.

$$\mathrm{BDI} = (\mathrm{MBT} + \mathrm{DBT}) / \mathrm{TBT} \qquad (1)$$

$$\mathrm{PDI} = (\mathrm{MPT} + \mathrm{DPT}) / \mathrm{TPT} \qquad (2)$$

If the BDI 1or PDI are >1, decomposition is progressing, and if they are <1, there has been a recent input of TBT. With the exception of the values at Stns. O3 and O4, the BDI values in the Harima Nada Sea and Osaka Bay were all >1. The BDI values at Stns. O3 and O4 were <0.7, suggesting that there had been a new input of TBT at these stations. The MBT and DBT concentrations at Stn. O3 and the DBT concentration at Stn. O4 were not determined. Since we set the value under the detection limit to 0 for the calculation of the BDI, it seems that even if a BDI value is <1, it cannot be concluded whether or not there has been a new input.

We compared the concentrations of each BT between the surface water and bottom water in the Harima Nada Sea and Osaka Bay (Fig. 2). In general, there was a trend for concentrations of TBT in water collected at the bottom to be higher than TBT concentrations collected from surface water, but these trends were not analyzed statistically because of the small number of data points. We used the BDI values to evaluate the inputs of TBT to the surface and bottom water samples. In all of the surface water samples, the BDI values were >1. In contrast, the BDI values in all of the bottom water samples except for that from Stn. H3 ranged from 0.28 to 0.93, and the BDI value at Stn. H3 was near 1, suggesting the possibility that TBT had been mobilized from sediment into the bottom water. No PT compounds were detected in water samples from the Harima Nada Sea or Osaka Bay (Table 3).

Sediment samples

The concentrations of TBT in the sediment samples from the Harima Nada Sea and Osaka Bay were in the range of 5.5–17 ng/g dw and 2.0–28 ng/g dw, respectively (Table 5). After the worldwide ban, the concentrations of TBT in the sediment samples were in the range of <0.3–1.980 ng/g dw as shown as Table 4. The concentrations of TBT in the sediment collected from the Harima Nada Sea and Osaka Bay were lower than these reported values. However, the background concentrations of TBT in sediment from a French aquatic environment ranged from <0.25 to 1.16 ng/g dw (Cavalheiro et al. 2016). The TBT values that we measured in the present study were higher than the background levels in France. On the other hand, Harino et al. (1998) observed that the TBT concentrations in sediment samples from the Port of Osaka before the worldwide TBT ban were 10–2,100 ng/g dw , indicating that the input of TBT decreased due to the AFS convention.

Table 5 lists the concentrations of ΣBTs in the present sediment samples. The concentrations of ΣBTs in the Harima Nada Sea and Osaka Bay ranged from 44 to 1,900 ng/g dw and 200 to 370 ng/ng dw, respectively. Our comparison of the TBT concentrations detected in sediment samples from the Harima Nada Sea with those in the sediment samples from Osaka Bay revealed no statistical significant differences in TBT concentrations between the two regions. The reported concentrations of ΣBTs in sediment samples collected after the worldwide ban are 7.9–2,120 ng/g dw, and the ΣBT concentrations in the present study's sediment samples from the Harima Nada Sea and Osaka Bay are thus similar to the concentrations reported in other areas.

We also investigated the horizontal distribution of ΣBTs in the Harima Nada Sea and Osaka Bay. The concentrations of ΣBTs were high at Stn. H4 in the Harima Nada Sea and Stns. O1 and O2 in Osaka Bay. We thus suspect that these sampling stations were once heavily contaminated by OT compounds. Moreover, the water depth at Stn. H4 is 40 m, which is greater than the depth at the other stations. The degradation of TBT was reported to be slower under anaerobic conditions (de Mora et al. 1989; Dowson et al. 1993). Since fishing is the main industry around Stn. H4, it is likely that many fishing boats sailed in the vicinity of the station, which would lead to a high load of TBT.

As shown in Figure 1, Stn O1 is located at the mouth of the large Yodo River, and because this area is used as an international trading port, there are many factories around the station. Industry is also the main activity around Stn. O2, which is surrounded by trading ports and international airports. It is likely that chemical pollutants flow from Stn. O1 to Stn. O2, since this is the direction of the water flow in Osaka Bay. It is thus unsurprising that the concentrations of ΣBTs were high at these sampling sites.

The concentrations of TPT in sediment samples from the Harima Nada Sea and Osaka Bay were in the range of <0.1 to 2,700 ng/g dw and 0.2 to 1,300 ng/g dw, respectively (Table 5). Although there are few reports on TPT concentrations after the worldwide ban, their present values were <0.5–346 ng/g dw (Table 4). Judging from the reported values, the concentrations of TPT in the Harima Nada Sea and Osaka Bay are observed the higher trend than the previously reported values.

The concentrations of ΣPTs (the sum of the MPT, DPT, and TPT concentrations) in the sediment samples from the Harima Nada Sea and Osaka Bay ranged from 8.3 to 2,700 ng/g dw and 6.0 to 1,300 ng/g dw, respectively. Few recent studies measured all three (MPT, DPT, and TPT) concentrations. It was reported that ΣPTs were not below the detection limit in most samples from seaports on the Gulf of Gdansk on the southern Baltic coast, and the maximum concentration of ΣPTs in the samples was 660 ng/g dw (Table 4), indicating that the concentrations of ΣPTs in the Harima Nada Sea and Osaka Bay were the fairly high.

We next consider the horizontal distribution of ΣPTs. The highest concentrations of ΣPTs were observed at. Stn. H8 in the Harima Nada Sea and Stns. O1 and O2 in Osaka Bay. The main industries at Stn. H8 are tourism and fishing. Higher concentrations of ΣPTs in sediment were observed not only in the industrial and fishing areas but also in the tourist areas of all three stations. The horizontal distribution of TPT was similar to that if the ΣPTs.

With regard to BT compounds, we observed that the horizontal distributions of ΣBTs and TBT differed, but in the case of PT compounds, the horizontal distributions of TPT were similar to those of the ΣPTs. This may be due to the difference in the decomposition of TBT and TPT in sediment. In addition, about half of the PDI values at each station were ≤1. This demonstrates that there was a new input of TPT into the sediment. Concerning the possible reasons for this, we speculate that TPT was used as a pesticide.

Alternative biocides

Seawater samples

We compared the concentrations of each alternative biocide in the surface water samples between the Harima Nada Sea and Osaka Bay and with the reported values. The horizontal distributions of each alternative biocide in the Harima Nada Sea and Osaka Bay and the effect of antifouling biocide for aquatic organisms were also determined.

As shown in Table 6, diuron in surface water samples from the Harima Nada Sea and Osaka Bay was detected in the ranges of <1–5.6 ng/L and <1–53 ng/L respectively, and the levels of diuron in the Harima Nada Sea were statistic significantly lower than those in Osaka Bay by statistical analysis.. As shown in the concentrations of diuron worldwide have ranged from <0.1 to 70 ng/L (Table 7). In the Harima Nada Sea and Osaka Bay, the levels of diuron were lower than the levels detected elsewhere in the world. We compared the diuron levels identified in the present study with those in water samples collected from the Port of Osaka before the ban of diuron by the IMO: 0.8–267 ng/L (Harino et al. 2005). The diuron concentrations that we observed are lower than those documented before the ban.

We also compared the diuron concentrations in the Harima Nada Sea and Osaka Bay with the acute toxicity levels for aquatic organisms. The reported lethal concentration (LC_{50}) values for Crustacea and fish are >3,000 μg/L and >7,800 μg/L, respectively (Bao et al. 2011). Nebeker and Schuytema (1998) stated that the NOAEL (no observed adverse effect level) values for freshwater cladocerans, amphipods, midges, minnows, worms, and snails ranged from 1,800 to 20,000 μg/L. The NOEC (no observed effect concentration) for pacific oyster (*Crassostrea gigas*) embryos was 9,500 ng/L (Onduka et al. 2022). The diuron concentrations in the present study's water samples from the Harima Nada Sea and Osaka Bay are thus lower than the values associated with the toxicity levels for aquatic organisms.

The Sea-Nine 211 in surface water samples from the Harima Nada Sea was in the range <1–1.8 ng/L, but Sea-Nine 211 was not detected in any of the water samples from Osaka Bay (Table 6).

There have been few reports concerning the concentrations of Sea-Nine 211 (Table 7). The concentrations of this compound in other aquatic areas has ranged from <0.1 to 31 ng/L. We observed that the Sea-Nine 211 levels in the Harima Nada Sea were lower than those in other aquatic areas.

We next compared these values with the concentration of Sea-Nine in water from the Port of Osaka before the IMO ban of TBT. Before the ban, the concentrations of Sea-Nine 211 were in the range <3–4 ng/L (Harino et al. 2005), and the Sea-Nine 211 concentrations identified in the present investigation are thus lower than those before the ban.

We compared the Sea-Nine 211 concentrations in the Harima Nada Sea and Osaka Bay with the acute toxicity levels for aquatic organisms. It was reported that the 72-hr LC_{50} for Crustacea were over 0.42–4 μg/L (Femandez-Alba et al. 2002; Myers et al. 2006). Onduka et al. (2022) reported that the NOEC for Pacific oyster (*C. gigas*) embryos was <3 ng/L; the Sea-Nine 211 concentrations detected in the Harima Nada Sea and Osaka Bay were thus lower than the LC_{50} values. The chronically toxic levels of Sea-Nine 211 for crustaceans (*Daphnia magna*) and fish (*Onorhynchus tshawytcha*) have ranged from 1,200 ng/L to 14,000 ng/L (Shade et al. 1994; Okamura et al. 2002). Mochida et al (2010) reported that 96-hr LC_{50} values for Red Sea bream and the fish *Fundulus heteroclitus* (mummichog) were 5,100 and 4,700 ng/L, respectively. It was shown that the lowest reported 72-hr half-maximal effective concentration (EC_{50}) of Sea-Nine 211 is 160 ng/L for the alga *Skeletonema costatum* and that this level of Sea-Nine 211 directly disrupts cell division or biosynthetic pathways related to cell division (Mochida et al. 2015). Onduka et al. (2013) reported the 72-hr NOEC values of Sea-Nine 211 in the following algae: *Dunaliella tertiolecta*, 1,200 - 1,700 ng/L; *Tetraselmis tetrathele*, 180 - 480 ng/L; *Chaetoceros calcitrans*, 40 - 120 ng/L; and *Skeletonema costatum*, 60 - 70 ng/L and the 24-hr EC_{50} values for the crustaceans *Tigriopus japonicus* (1,400–1,800 ng/L) and

Portunus trituberculantus (11,000–13,000 ng/L). The concentrations of Sea-Nine 211 which detected in our present analyses are thus lower than the values that are toxic to aquatic organisms.

Irgarol 1051 in the water samples from the Harima Nada Sea and Osaka Bay was detected in the ranges <1–2.5 ng/L and <1–4.0 ng/L, respectively (Table 6). The reported concentrations of Irgarol 1051 have been <0.1–55 ng/L (Table 7). The concentrations of Irgarol 1051 measured in the present study were thus lower than the reported values. We compared the Irgarol 1051 values in the water samples from the Harima Nada Sea and Osaka Bay with those in the water samples from the Port of Osaka before the ban of TBT by the IMO, i.e., <0.8–267 ng/L (Harino et al. 2005). The present Irgarol 1051 values are lower than those obtained before the TBT ban.

We also compared the Harima Nada Sea and Osaka Bay Irgarol 1051 concentrations with the acute toxicity levels for aquatic organisms. Mochida et al. (2019) researched the physiological effects of Irgarol 1051 on eelgrass (*Zostera marina*) and stated that a toxic effect on growth was observed at Irgarol 1051 concentrations ≥1,000 ng/L. Several research groups observed that the LC_{50} values for Crustacea and fish were >5,700 μg/L (Okamura et al. 2000, 2002; Toth et al. 1996; Bao et al. 2011). The reported NOEC for pacific oyster (*C. gigas*) embryos is 7,000 ng/L (Onduka et al. 2022). The Irgarol 1051 concentrations have also been compared with the endpoint of chronic toxicity to the growth and survival of a crustacean (*Mysidopsis bahia*) and fish (*Oncorhynchus mykiss*): the endpoint values ranged from 4,020 to 110,000 ng/L (Hall Jr. et al. 1999). Judging from the concentration of Irgarol 1501 detected in the Harima Nada Sea and Osaka Bay, there appears to be a very low possibility that the Irgarol 1051 in these regions would affect the aquatic organisms.

M1 is the degradation product of Irgarol 1051, and we observed M1 concentrations in the range 1.9–33 ng/L. The calculated ratio of M1 to Irgarol 1051 is in the range 1.2–34 ng/L, suggesting that the decomposition rate of Irgarol 1051 exceeded its input rate (Table 6). The reported concentrations of M1 have ranged from <3.2 to 63.4 ng/L (Table 7), and the M1 concentrations observed herein are similar to those observed in other aquatic areas. We compared these values with those in water samples from the Port of Osaka before the IMO ban of M1, i.e., <1.9–167 ng/L (Harino et al. 1998); the present M1 values are lower than those observed before the ban.

The Harima Nada Sea and Osaka Bay concentrations were compared to the acute toxicity levels for aquatic organisms. The reported 96-hr LC_{50} concentrations for the growth of microalgae are 73–83 μg/L (Gatidou and Thomaidis 2007), and the M1 concentrations that we obtained are lower than these LC_{50} values.

We detected 28–61 ng/L and <1–343 ng/L concentrations of dichlofluanid in the Harima Nada Sea and Osaka Bay water samples, respectively, and the concentrations in Osaka Bay were significantly higher than those in the Harima Nada Sea by statistical analysis (Table 6). There have been few reports concerning dichlofluanid values (Harino 2016). Harino and Yamato (2021) reported that the dichlofluanid concentrations in Japan's in Tanabe Bay were <0.1–44 ng/L. The present study's dichlofluanid values were thus higher than the previously observed values.

We compared the Harima Nada Sea and Osaka Bay dichlofluanid concentrations with the acute toxicity levels for aquatic organisms. Fernandez-Alba et al. (2002) reported that the 96-hr LC_{50} values for dichlofluanid in Crustacea were 133–1,050 μg/L, and Bellas (2006) reported that the 48-hr LC_{50} dichlofluanid values for mussels and sea urchins were 627 μg/L and 81 μg/L, respectively. The levels of dichlofluanid in the Harima Nada Sea and Osaka Bay were lower than the LC_{50} values for aquatic animals. The NOAEL for fish and invertebrate aquatic organisms ranged from 2.65 to 4.55 μg/L (UK 2016); the present study's dichlofluanid concentrations were thus lower than the values causing acute toxicity and lower than the NOAEL.

No chlorothalonil was detected in the water samples from the Harima Nada Sea, and chlorothalonil was detected in Osaka Bay only at Stn. O2, where its concentration was near the detection limit (Table 6). As shown in Table 7, the chlorothalonil concentrations in Tanabe Bay are approx. 8–26 ng/L (Harino and Yamato 2021), which is higher than the values in the Harima Nada Sea and Osaka Bay. The high concentrations of chlorothalonil in Tanabe Bay may be due to the use of chlorothalonil as a pesticide, because Tanabe Bay is surrounded by forest areas.

We compared the Harima Nada Sea and Osaka Bay concentrations of chlorothalonil with the acute toxicity levels for aquatic organisms. Bao (2011) showed that the 96-hr LC_{50} values for Crustacea were 69–110 μg/L, and Bellas (2006) reported that the 48-hr LC_{50} values for mussels and sea urchins were 8.7 μg/L and 6.6 μg/L, respectively. The 72-hr EC_{50} and 72-hr NOEC values of chlorothalonil for an alga (*S. costatum*) were 950 ng/L and 560 ng/L, respectively. The 24-hr EC_{50} of a crustacean (*T. japonicus*) was 16,000 ng/L, and the 96-hr LC_{50} of Kuruma prawn (*Marsupenaeus japonicus*), red sea bream (*Pagrus major*), and mummichog (*F. heteroclitus*) were 290,000 ng/L, 35,000 ng/L and 61,000 ng/L, respectively; the 8-week NOEC and LOEC (the lowest tested concentration that is significantly different from control) values for mummichog were 11,000 ng/L and 32,000 ng/L. The reported NOAELs of chlorothalonil for fish and invertebrates are 1,300 and 600 ng/L (Thistle and Durkin 2015). Onzuka et al. (2012) observed that in an early-live-stage test with mummichog embryos, the respective lowest- and no-observed-effect concentrations were 32,000 and 11,000 ng/L. Our analyses revealed that the concentrations of chlorothalonil in the Harima Nada Sea and Osaka Bay are lower than the reported toxicity levels for aquatic organisms.

As depicted in Figure 3, we also compared the concentration of each alternative biocide between the surface water and the bottom water. Although no statistical analysis was performed, the following trends were observed. The concentrations of diuron, Sea-Nine 211, and Irgarol 1051 in the surface-water samples were higher than those in the bottom-water samples. At six of 11 sampling sites, the dichlofluanid concentrations in the surface-water samples were also higher than those in the bottom-water samples. The data for the alternatives biocides showed the opposite trend compared to the OT data. Collectively, our results indicate that the detected alternative biocides were not re-mobilization from the sediment, but rather were eluted from ship hulls.

Our above-described findings can be summarized as follows. The concentrations of antifouling agents detected in the Harima Nada Sea and Osaka Bay were lower than those detected in other sea areas, with the exception of dichlofluanid. In terms of the reason why dichlofluanid was detected, we suspect that this compound may have been used as a pesticide in addition to its use as an antifouling paint on ship hulls. More specifically, the concentrations of dichlofluanid may have been higher than in other aquatic regions because greater amounts of dichlofluanid were used as a pesticide around the aquatic areas. Our analyses also revealed that the concentrations of alternative biocides in the Harima Nada Sea and Osaka Bay are not at levels that affect aquatic organisms.

Sediment samples

The concentrations of diuron in sediment were 32–488 ng/g dw (mean 117 ng/g dw) and 88–342 ng/g dw (mean 153 ng/g dw) in the Harima Nada Sea and Osaka Bay, respectively (Table 8) and statistical analysis showed no significant differences. We compared these values with the reported values in other sediment samples, which ranged from 0.01 to 1,112 ng/g dw; the the Harima Nada Sea and Osaka Bay concentrations of diuron are within the reported range (Table 9). We also compared these values with those in Port of Osaka sediment samples collected before the worldwide diuron ban. The concentration of TBT was in the range 10–2,100 ng/g dw (Harino et al. 2005), and the present study's diuron concentrations tended to be thus lower than those before the TBT ban, although no statistical analysis was performed.

The ranges of the Harima Nada Sea and Osaka Bay concentrations of Sea-Nine 211 in sediment were 47–591 ng/g dw (mean 135 ng/g dw) and 63–93 ng/g dw (mean 75 ng/g dw), respectively, with significantly higher concentrations in the Harima Nada Sea by statistical analysis (Table 8). There are few reports on Sea-Nine 211 concentrations in sediment samples (Table 9). Batista-Andrade et al. (2018) reported Sea-Nine 211 concentrations at <038–81.6 ng/g dw in sediment from Panama. Harino and Yamato (2021) noted that like diuron, no Sea-Nine 211 was detected in Tanabe Bay. The Harima Nada Sea and Osaka Bay concentrations of Sea-Nine 211 tended to be higher than the reported values, although no statistical analysis was performed.

We compared the Harima Nada Sea and Osaka Bay Sea-Nine 211 values with those in sediment samples from the Port of Osaka collected before the IMO's ban on TBT: 10–2,100 ng/g dw (Harino et al. 1998). The concentrations of Sea-Nine 211 in the present investigation are lower than those obtained before the ban. In their study of the alga *C. calcitrans*, Onduka et al. (2013) observed a 14-day LC_{50} and 14-day NOEC of growth at 110 ng/g dw and 9.7 ng/g dw, respectively; the lowest 72-hr NOEC value was 0.04 ng/g dw. Because the concentrations of Sea-Nine 211 were higher than the NOEC for alga, there is concern about the impact of Sea-Nine 211 on benthic organisms.

The Harima Nada Sea and Osaka Bay concentrations of Irgarol 1051 showed no significant differences by statistical analysis, ranging from 33 to 128 ng/g dw (mean 57 ng/g dw) and from 43 to 83 ng/g dw (mean 56 ng/g dw), respectively (Table 8). The reported values of Irgarol 1051 have been

<0.08–1,112 ng/g dw (Table 9). Although the concentrations of Irgarol 1051 in the Harima Nada Sea and Osaka Bay are lower than those recorded in the Seto Inland Sea, they are higher than those in the other areas. We compared these values with those in sediment samples from the Port of Osaka obtained before the TBT ban by the IMO, which range from 10 to 2,100 ng/g dw (Harino et al.1998). The concentrations of Irgarol 1051 that we obtained in the present study are thus lower than those the before ban.

As shown in Table 8, the Harima Nada Sea and Osaka Bay concentrations of M1 were 60–128 ng/g dw (mean 137 ng/g dw) and 104–377 ng/g dw (mean 174 ng/g dw). Although the Irgarol 1051 concentrations in the Harima Nada Sea were similar to those in Osaka Bay, the M1 concentrations in Osaka Bay were significantly higher than those in the Harima Nada Sea by statistical analysis. Harino and Yamato (2021) detected no M1 in Tanabe Bay. The present study's detection of M1 at relatively high concentrations is noteworthy.

The respective Harima Nada Sea and Osaka Bay concentrations of dichlofluanid ranged from 67 to 8,038 ng/g dw (mean 1,130 ng/g dw) and from 104 to 263 ng/g dw (mean 162 ng/g dw), and those concentrations of dichlofluanid in the Harima Nada Sea were higher than those in Osaka Bay by statistical analysis. (Table 8). Dichlofluanid was not detected in sediment from Panama (Batista-Andrade et al. 2018), and Harino and Yamato (2021) reported that dichlofluanid was also not detected in Tanabe Bay (Table 9). The concentrations of dichlofluanid in the Harima Nada Sea and Osaka Bay are in contrast to the absence of dichlofluanid in Tanabe Bay, suggesting the past use of chlorothalonil as a pesticide.

As shown in table 8, the results of our analyses demonstrated chlorothalonil concentration ranges at 31–2,975 ng/g dw (mean 428 ng/g dw) in the Harima Nada Sea and 49–165 ng/g dw (mean

83 ng/g dw) in Osaka Bay (significantly higher in the Harima Nada Sea by statistical analysis). There have been only a few studies of chlorothalonil in seawater. The study by Harino and Yamato (2021) revealed that the chlorothalonil concentrations in Tanabe Bay ranged from <0.1 to 8.2 ng/g dw. Our findings demonstrate that the concentrations of alternative biocides in sediment from the Harima Nada Sea and Osaka Bay tended to be higher than those in Tanabe Bay. Similar to the water samples, the reason for this differencee may be related to the discharge of chlorothalonil used as a pesticide to the sea areas.

The above-described results can be summarized as follows. Although the concentrations of alternative biocides in sediment samples in this study were generally lower than those in other aquatic areas, the concentrations of these compounds in sediment were higher than those in the other aquatic areas. In regard to the horizontal distribution, although there was no major difference in the concentrations of alternative biocides concentrations in the water samples collected in this study, the concentrations of most of the alternative biocides in the sediment samples from Stns. H1, H2, and O1 (which were in proximity to the fishing and industrial areas) were higher than those at the other stations (Tables 5, 6). This trend indicates that the use of alternative biocides is currently decreasing, although in the past alternative biocides were widely used on ship hulls. In addition, the concentrations of most of the alternative biocides were lower than those before the use of TBT was banned by the IMO, suggesting that (*i*) the alternative biocides measured in this study are no longer in use, and (*ii*) their compounds may have been replaced by other alternative compounds such as pyrithions and borans.

Conclusions

Although the concentrations of ΣBT and ΣPT in water samples collected from Japan's Harima Nada Sea and Osaka Bay were lower than those in the other sea areas, the concentrations of ΣBT and ΣPT in sediment from these areas were higher than those in the other areas. These differences indicate that the Harima Nada Sea and Osaka Bay have been contaminated heavily by OTs in the past. We speculate that the detection of TBT in water samples from these areas is due to re-mobilization from sediment. Although the concentrations of alternative biocides in the water samples from the Harima Nada Sea and Osaka Bay were lower than the values obtained in the previously reported areas (with the exception of dichlofluanid), the concentrations in sediment from the present areas were higher. We thus suspected that the Harima Nada Sea and Osaka Bay areas were heavily contaminated by alternative biocides both in past.

It can also be inferred from the results of our comparison of the alternative biocide concentrations between surface water and bottom water that the origin of the new input of alternative biocides is due to the use of antifouling biocides and pesticides. We suspect that the OT contamination persists in closed sea areas via re-mobilization from sediment, and we observed that the TBT levels were higher than those causing imposex in gastropods. We also detected alternative biocides in water and sediment samples, although their concentrations were lower than the levels that are toxic to aquatic organisms.

Further research is necessary to measure the concentrations of antifouling biocides in a greater number of samples collected from the various bays that are located in the Seto Inland Sea. Based on those findings, we hope to clarify the reasons why the concentrations of antifouling biocides differ among various bays by conducting the appropriate statistical analyses plus evaluations of the effects of the antifouling biocides on aquatic organisms. Dredging should also be considered in the cases of heavily contaminated sediments.

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Statements and Declarations

All authors contributed to the study conception and design. The sample collection were performed by Madoka Ohji, Kumiko Kono, Toshimitsu Onzuka, Takashi Hano and Kazuhiko Mochida. Data analysis were performed by Hiroya Harino. The first draft of the manuscript was written by Hiroya Harino and all authors commented on the manuscript.

Figure Captions

Fig. 1. The map of sampling sites at the Harima Nada Sea and Osaka Bay, Japan.

Arrows indicate the flow of ocean currents.

Fig. 2. The horizontal distribution of butyltin compounds in the samples of surface (S) water and bottom (B) water. The numbers in parentheses are the butyltin degradation index (BDI) values.

Fig. 3. The horizontal distribution of alternative biocides in the samples of surface (S) water and bottom (B) water.

Table 1. Overview of sampling sites

Table 2. The abbreviations and mass numbers of the antifouling biocides examined in this study

Table 3. The concentrations of butyltin compounds in water samples collected in 2017

Table 4. The concentrations of OTs in water and sediment samples collected globally and reported in the peer-reviewed literature

Table 5. The concentrations of butyltin and phenyltin compounds in sediment samples collected in 2018

Table 6. The concentrations of alternative biocides in water samples collected in 2017

Table 7. The concentrations of alternative biocides in water samples collected globally and reported in the peer-reviewed literature

Table 8. The concentrations of alternative biocides in sediment samples collected in 2018

Table 9. The concentrations of alternative biocides in sediment samples collected globally and reported in the peer-reviewed literature

Fig.1

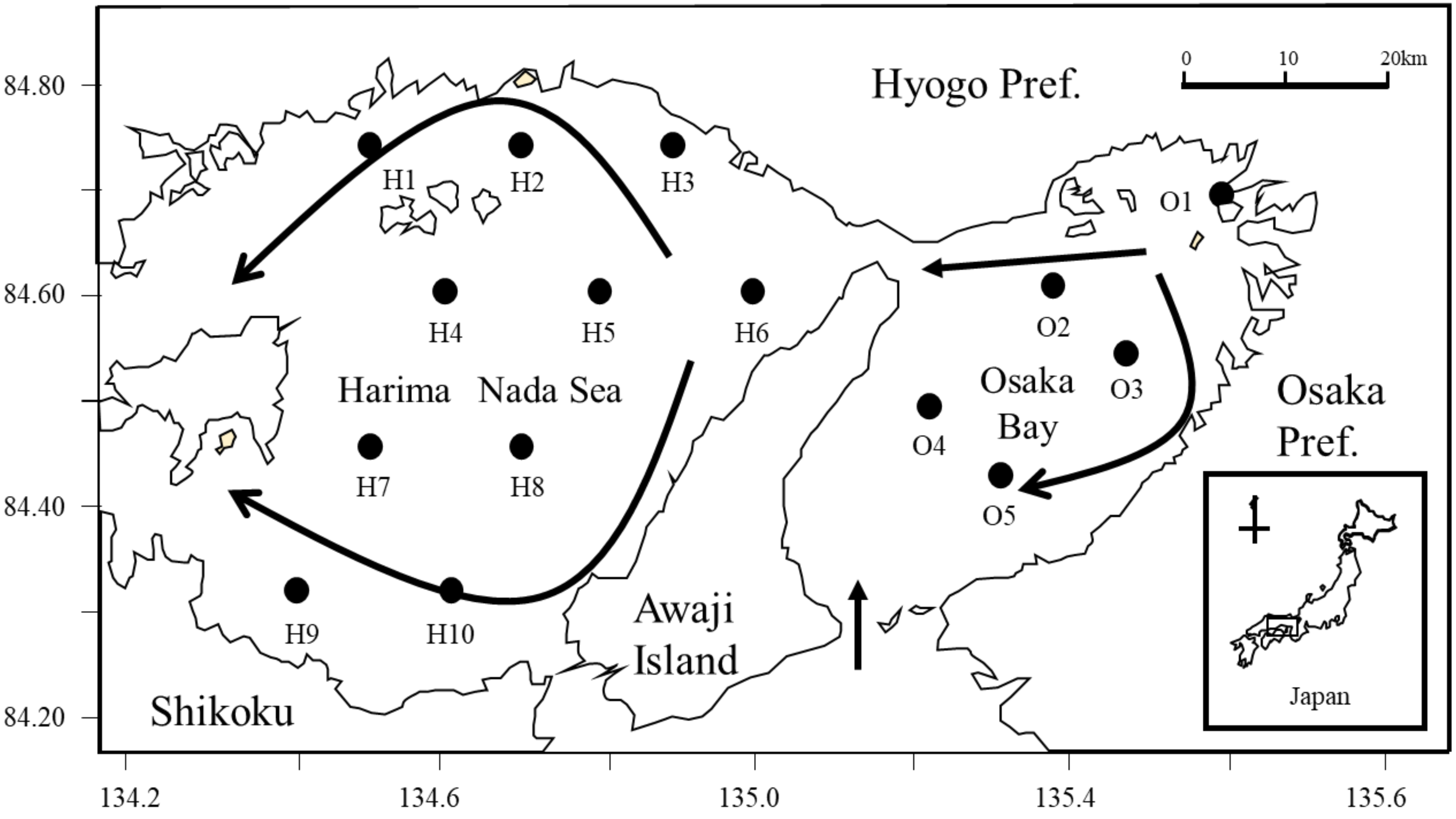
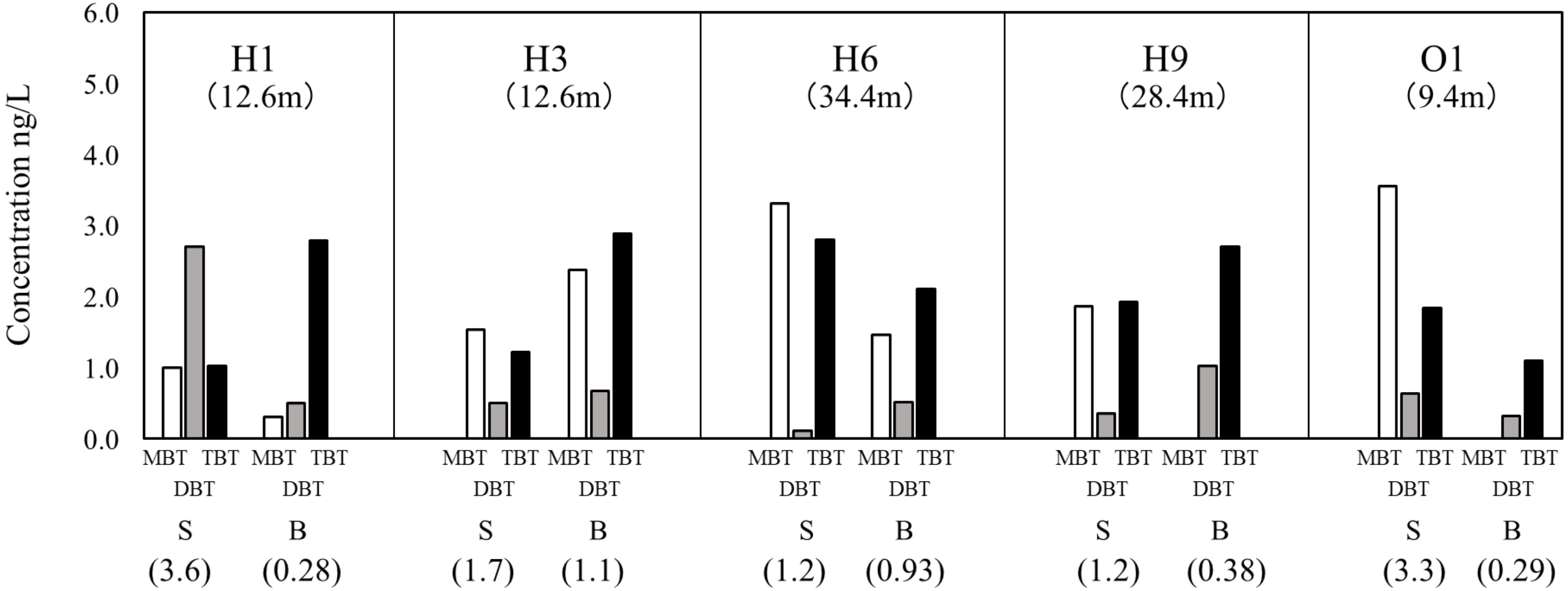

84.80
84.60
84.40
84.20
134.2
134.6
135.0
135.4
135.6
Hyogo Pref.
0
10
20km
H1
H2
H3
O1
H4
H5
H6
O2
Harima Nada Sea
Osaka Bay
O3
O4
O5
Osaka Pref.
H7
H8
Japan
H9
H10
Awaji Island
Shikoku

Fig.2



Concentration ng/L
6.0
5.0
4.0
3.0
2.0
1.0
0.0
H1
(12.6m)
H3
(12.6m)
H6
(34.4m)
H9
(28.4m)
O1
(9.4m)
MBT TBT MBT TBT
DBT DBT
S B
(3.6) (0.28)
MBT TBT MBT TBT
DBT DBT
S B
(1.7) (1.1)
MBT TBT MBT TBT
DBT DBT
S B
(1.2) (0.93)
MBT TBT MBT TBT
DBT DBT
S B
(1.2) (0.38)
MBT TBT MBT TBT
DBT DBT
S B
(3.3) (0.29)

Fig.3

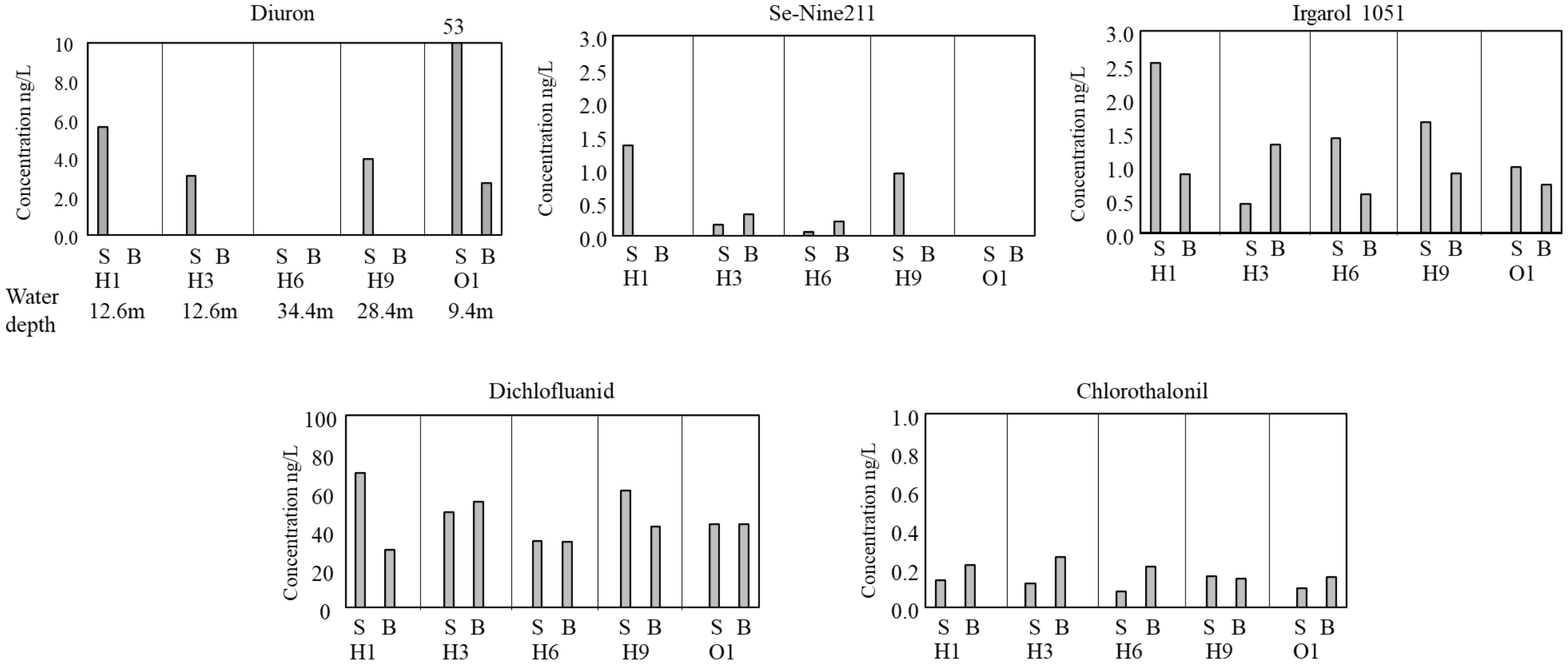

Diuron
53
Concentration ng/L
10
8.0
6.0
4.0
2.0
0.0
S B
H1
H3
H6
H9
O1
Water depth
12.6m
12.6m
34.4m
28.4m
9.4m
Se-Nine211
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Irgarol 1051
Dichlofluanid
100
80
60
40
20
0
Chlorothalonil
1.0
0.8
0.6
0.4
0.2
0.0

Table 1

Table 1 Overview of sampling sites

Sampling site		Latitude	Longitute	Water samples		Sediment samples		Activities
				Sampling date	Water depth (m)	Sampling date	Ignition loss (%)	
Harima Nada Sea	H1	34-43.0N	134-27-0E	11,Sep.,2017	13.6	30,Nov.,2018	10.3	Fishery
	H2	34-43.0N	134-37.0E	11,Sep.,2017	21.7	30,Nov.,2018	5.3	Industry, Fishiery
	H3	34-43.0N	134-47.0E	10,Sep.,2017	13.6	30,Nov.,2018	4.2	Industry
	H4	34-35.0N	134-32.0E	11,Sep.,2017	40.7	29,Nov.,2018	7.0	Fishery, Tourist business
	H5	34-35.0N	134-42.0E	09,Sep.,2017	24.1	29,Nov.,2018	6.0	Fishery, Tourist business
	H6	34-35.0N	134-52.0E	10,Sep.,2017	35.4	29,Nov.,2018	0.9	Fishery, Tourist business
	H7	34-27.0N	134-27.0E	09,Sep.,2017	40.7	29,Nov.,2018	8.4	Fishery, Tourist business
	H8	34-27.0N	134-37.0E	09,Sep.,2017	33.8	29,Nov.,2018	8.6	Fishery, Tourist business
	H9	34-19.0N	134-22.0E	09,Sep.,2017	29.4	28,Nov.,2018	7.6	Fishery
	H10	34-19.0N	134-32.0E	09,Sep.,2017	33.8	29,Nov.,2018	3.9	Fishery
Osaka bay	O1	34-40.0N	135-22.5E	10,Sep.,2017	10.4	30,Nov.,2018	3.9	Industry, International trading port
	O2	34-35-2N	135-11.8E	09,Sep.,2017	20.9	29,Nov.,2018	6.8	Industry, International Airport
	O3	34-31.4N	135-16.5E	10,Sep.,2017	19.2	30,Nov.,2018	8.6	Industry, Fishery
	O4	34-28.6N	135-03.5E	10,Sep.,2017	23.4	30,Nov.,2018	6.8	Fishery, Tourist business
	O5	34-24.8N	135-08.2E	10,Sep.,2017	53.9	30,Nov.,2018	6.9	Fishery,

Table 2

Table 2 The abbreviation and mass numbers of the antifouling biocides examined in this study

Name	Abbreviation	Q1	Q2
Monobutyltin	MBT	235	233
Dibutyltin	DBT	261	263
Tributyltin	TBT	263	261
Monophenyltin	MPT	253	255
Diphenyltin	DPT	303	301
Triphenyltin	TPT	351	349
3-(3,4-dichlorophenyl)-1,1-dimethylurea	Diuron	187	159
4,5-dichloro-2-n-octyl-4-isothiazolin-3-one	Sea-Nine 211	246	281
2-methylthio-4-tert -butylamino-6-cyclopropylamino-s-triazine	Irgarol 211	182	253
2-methylthio-4-tert -butylamino-6-amino-s-triazine	M1	157	213
N,N-dimethyl-N'-phenyl-N'-(dichlorofluoromethylthio) sulfamide	Dichlofluanide	224	332
2,4,5,6-Tetrachloroisophthalonitrile	Chlorothalonil	266	264

Table 3

Table 3 The concentrations of butyltin compounds in water samples collected in 2017

	Station	MBT	DBT	TBT	Total BTs	BDI	MPT	DPT	TPT	Total PTs	PDI
Harima Nada Ssa	H1	1.0	2.7	1.0	4.7	3.7	<1.0	<1.0	<1.0	<1.0	-
	H2	2.0	1.0	1.5	4.2	2.0	<1.0	<1.0	<1.0	<1.0	-
	H3	1.5	1.0	1.2	3.2	2.1	<1.0	<1.0	<1.0	<1.0	-
	H4	1.8	1.0	1.9	4.5	1.5	<1.0	<1.0	<1.0	<1.0	-
	H5	8.1	1.0	1.7	10	5.4	<1.0	<1.0	<1.0	<1.0	-
	H6	3.3	<1	2.8	6.3	1.2	<1.0	<1.0	<1.0	<1.0	-
	H7	5.5	<1	1.4	7.0	3.9	<1.0	<1.0	<1.0	<1.0	-
	H8	1.9	<1	1.0	3.1	1.9	<1.0	<1.0	<1.0	<1.0	-
	H9	1.9	<1	1.9	4.1	1.0	<1.0	<1.0	<1.0	<1.0	-
	H10	<1	<1	1.3	1.6	-	<1.0	<1.0	<1.0	<1.0	-
	Average	2.7	0.7	1.6	5.0	2.1	<1.0	<1.0	<1.0	<1.0	-
Osaka Bay	O1	3.6	1.0	1.8	6.0	2.6	<1.0	<1.0	<1.0	<1.0	-
	O2	2.5	<1	1.4	4.2	1.7	<1.0	<1.0	<1.0	<1.0	-
	O3	<1	<1	1.0	1.6	0.0	<1.0	<1.0	<1.0	<1.0	-
	O4	1.4	<1	2.0	3.7	0.7	<1.0	<1.0	<1.0	<1.0	-
	O5	5.0	1.0	2.2	7.8	2.7	<1.0	<1.0	<1.0	<1.0	-
	Average	2.5	<1	1.7	4.2	1.5	<1.0	<1.0	<1.0	<1.0	-

ng/L

Table 4

Table 4 The concentrations of OTs in water and sediment samples collected globally and reported in the peer-reviewed literature

Samples	Region	TBT	ΣBTs	TPT	ΣPTs	Ref
Water samples (ng/L)	Hsiao Liouciou Island, Taiwan	6.36 - 76.83	34.2-190			Liu et al. (2011)
	Three Gorges Reservoir region in China	<0.35 - 393.35	<1.13-487.27			Gao et al. (2017)
	Tanabe Bay	4 - 28				Harino and Yamato (2021)
	Harima Nada Sea	1.0 – 2.8	1.6 – 10			This study
	Osaka Bay	1.0 – 2.2	1.6 – 7.8			This study
Sediment samples (ng/g dw)	The ports of Gidnsk and Gdnya	<0.3 – 1,980		<0.5 – 346		Filipkowska et al. (2011)
	the Gulf of Gdansk on the southern Baltic coast				ND – 660	Filipkowska et al. (2011)
	the port of Gdynia, Poland		2,120			Radke et al. (2012)
	Futian Mangrove Wetland in Shenzhen, China	2.42		1-10		Deng et al. (2015)
	the Yangtze estuary and the adjacent region of East China					Chen et al. (2017)
	Luchao Port	<1 – 9.22	4.10 – 24.48			Chen et al. (2017)
	Yangsham Port	<1 – 10.8	4.28 – 26.86			Chen et al. (2017)
	Yangtze estuary	<1 – 15.7	9.97 - 50.1			Chen et al. (2017)
	coastal area of Koster, Sweden		500			Egardr et al. (2017)
	Panama	<1 - 149	25 - 227			Batista-Andrade et al. (2018)
	the coastal zone of the Mediterranean Sea		7.9 - 1,362			Turk et al. (2020)
	Sri Lanka	17 - 107				Bandara et al. (2021)
	the Atlantic (Ria de Vigo)	4.10 - 75.4				Concha-Grafia et al. (2021)
	Mediterranean (Mar Menor lagoon)	5.65 - 93.4				Concha-Grafia et al. (2021)
	Tanabe Bay			2-37		Harino and Yamato (2021)
	Harima Nada Sea	5.5 - 17	44 – 1,900	<0.1-2,700	8.3-2,700	This study
	Osaka Bay	2.0 - 28	200 – 370	0.2-1,300	6.0-1,300	This study

Table 5

Table 5 The concentrations of butyltin and phenyltin compounds in sediment samples collected in 2018

	Station	MBT	DBT	TBT	Σ BT	BDI	MPT	DPT	TPT	ΣTPT	PDI
Harima Nada Ssa	H1	58	25	6.4	89	13	5.8	0.40	0.37	21	40
	H2	57	39	14	110	6.9	20	0.17	0.51	21	40
	H3	22	16	5.5	44	6.9	20	0.53	0.45	21	45
	H4	800	1,100	8.4	1,900	230	7.5	0.36	0.38	8.3	21
	H5	29	8.2	8.2	45	4.5	9.2	0.41	0.43	10	23
	H6	13	50	7.5	71	8.4	12	0.37	<0.1	12	-
	H7	69	14	17	100	4.9	20	0.56	250	270	0.10
	H8	52	11	6.5	70	9.6	13	0.44	2,700	2,700	<0.10
	H9	59	18	15	92	5.1	9.9	0.61	480	490	<0.10
	H10	25	13	12	50	3.2	6.7	0.42	1,200	1,200	<0.10
	Average	120	130	11	250	31	13	0.43	520	470	-
Osaka Bay	O1	140	180	28	350	11	4.6	0.32	1,300	1,300	<0.10
	O2	82	280	12	370	30	12	0.44	0.24	13	53
	O3	92	180	13	290	22	7.3	0.44	590	600	<0.10
	O4	38	140	19	200	9.3	11	0.33	44	55	0.25
	O5	<0.1	200	2.0	200	100	5.4	0.37	0.21	6.0	28
	Average	70	200	15	282	35	8.1	0.38	380	390	16

ng/g dw

Table 6

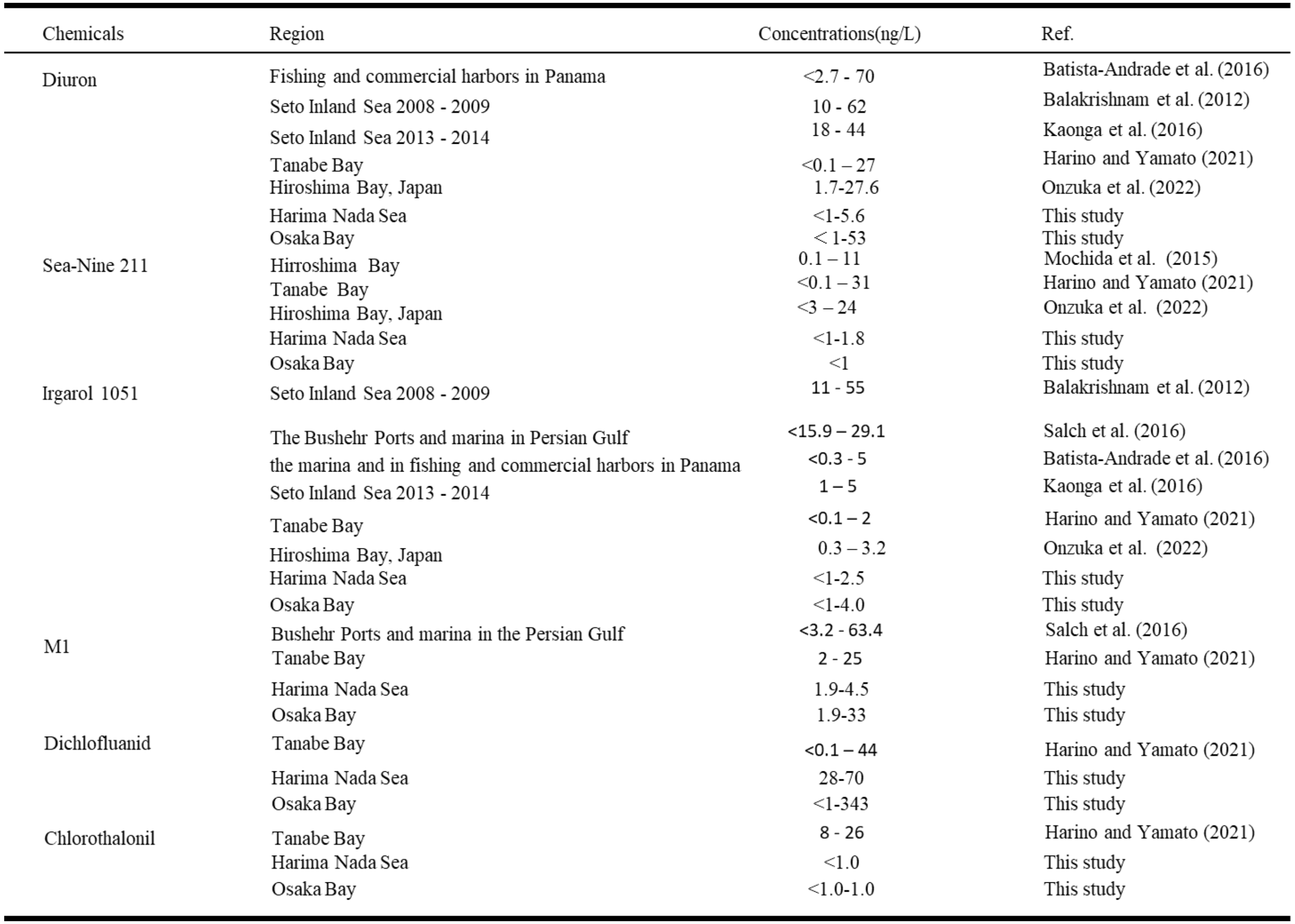
Table 6 The concentrations of alternative biocides in water samples collected in 2017

		Diuron	Sea-Nine211	Irgarol 1051	M1	$\frac{\text{M1}}{\text{Irgarol 1051}}$	Dichlofluanid	Chlorothalonil
Harima Nada Ssa	H1	5.6	1.3	2.5	4.5	1.8	70	<1.0
	H2	3.0	<1.0	<1	1.9	-	28	<1.0
	H3	3.1	<1.0	<1	3.9	-	50	<1.0
	H4	<1.0	1.8	1.3	2.5	1.9	46	<1.0
	H5	<1.0	1.0	1.0	2.6	2.6	61	<1.0
	H6	<1.0	<1.0	1.4	3.7	2.6	35	<1.0
	H7	<1	<1.0	1.0	2.6	3.0	45	<1.0
	H8	2.9	<1	1.0	2.5	2.5	41	<1.0
	H9	4.0	1.0	1.6	3.4	2.1	61	<1.0
	H10	1.3	<1.0	<1.0	2.6	-	41	<1.0
	Average	2.0	<1.0	1.0	3.0	1.7	48	<1.0
Osaka Bay	O1	53	<1.0	1.0	33	34	43	<1.0
	O2	12	<1.0	4.0	17	4.3	343	1.0
	O3	6.2	<1.0	1	4.8	9.3	53	<1.0
	O4	<1	<1.0	2.8	3.5	1.2	55	<1.0
	O5	<1.0	<1.0	<1.0	1.9	-	<1.0	<1.0
	Avetage	14	<1.0	1.8	12	10	99	<1.0

ng/L

Table 7

Table 7 The concentrations of alternative biocides in water samples collected globally and reported in the peer-reviewed literature



Chemicals	Region	Concentrations(ng/L)	Ref.
Diuron	Fishing and commercial harbors in Panama	<2.7 - 70	Batista-Andrade et al. (2016)
	Seto Inland Sea 2008 - 2009	10 - 62	Balakrishnam et al. (2012)
	Seto Inland Sea 2013 - 2014	18 - 44	Kaonga et al. (2016)
	Tanabe Bay	<0.1 – 27	Harino and Yamato (2021)
	Hiroshima Bay, Japan	1.7-27.6	Onzuka et al. (2022)
	Harima Nada Sea	<1-5.6	This study
	Osaka Bay	< 1-53	This study
Sea-Nine 211	Hirroshima Bay	0.1 – 11	Mochida et al. (2015)
	Tanabe Bay	<0.1 – 31	Harino and Yamato (2021)
	Hiroshima Bay, Japan	<3 – 24	Onzuka et al. (2022)
	Harima Nada Sea	<1-1.8	This study
	Osaka Bay	<1	This study
Irgarol 1051	Seto Inland Sea 2008 - 2009	11 - 55	Balakrishnam et al. (2012)
	The Bushehr Ports and marina in Persian Gulf	<15.9 – 29.1	Salch et al. (2016)
	the marina and in fishing and commercial harbors in Panama	<0.3 - 5	Batista-Andrade et al. (2016)
	Seto Inland Sea 2013 - 2014	1 – 5	Kaonga et al. (2016)
	Tanabe Bay	<0.1 – 2	Harino and Yamato (2021)
	Hiroshima Bay, Japan	0.3 – 3.2	Onzuka et al. (2022)
	Harima Nada Sea	<1-2.5	This study
	Osaka Bay	<1-4.0	This study
M1	Bushehr Ports and marina in the Persian Gulf	<3.2 - 63.4	Salch et al. (2016)
	Tanabe Bay	2 - 25	Harino and Yamato (2021)
	Harima Nada Sea	1.9-4.5	This study
	Osaka Bay	1.9-33	This study
Dichlofluanid	Tanabe Bay	<0.1 – 44	Harino and Yamato (2021)
	Harima Nada Sea	28-70	This study
	Osaka Bay	<1-343	This study
Chlorothalonil	Tanabe Bay	8 - 26	Harino and Yamato (2021)
	Harima Nada Sea	<1.0	This study
	Osaka Bay	<1.0-1.0	This study

Table 8

Table 8 Concentrations of alternative biocides in sediment samples collected in 2018

	Station	Diuron	Sea-Nine211	Irgarol1051	M1	$\frac{\text{M1}}{\text{Irgarol 1051}}$	Dichrofluanid	Chlorotharonil
Harima Nada Ssa	H1	116	122	47	73	1.5	8038	2975
	H2	488	591	128	541	4.2	2218	881
	H3	56	66	33	71	2.2	67	44
	H4	67	62	52	94	1.8	94	57
	H5	120	80	43	101	2.3	192	65
	H6	32	111	38	74	1.9	211	51
	H7	78	101	63	134	2.1	75	57
	H8	66	108	77	121	1.6	199	65
	H9	84	62	54	102	1.9	129	57
	H10	64	47	35	60	1.7	76	31
	Average	117	135	57	137	2.4	1130	428
Osaka Bay	O1	342	93	83	377	4.5	263	165
	O2	88	70	53	116	2.2	104	66
	O3	100	63	44	104	2.4	192	49
	O4	134	78	43	121	2.8	142	66
	O5	99	73	55	152	2.8	111	70
	Average	153	75	56	174	3.1	162	83

ng/g dw

Table 9

Table 9 The concentrations of alternative biocides in sediment samples collected globally and reported in the peer-reviewed literature

Chemicals	Region	Concentrations (ng/g dw)	Ref.
Diuron	Seto Inland Sea 2008 - 2009	0.01 – 0.09	Balakrishnam et al. (2012)
	Seto Inland Sea 2013 - 2014	682 – 1,112	Kaonga et al. (2016)
	Fishing and commercial harbors in Panama	<0.75 – 14.1	Gatista-Andrade. (2018)
	Tanabe Bay	<0.1	Harino and Yamato (2021)
	Harima Nada Sea	<1-5.6	This study
	Osaka Bay	< 1-53	This study
Sea-Nine 211	Fishing and commercial harbors in Panama	<0.38 – 81.6	Batista-Andrade. (2018)
	Tanabe Bay	<0.1	Harino and Yamato (2021)
	Harima Nada Sea	47-591	This study
	Osaka Bay	63-93	This study
Irgarol 1051	Seto Inland Sea 2008 - 2009	11 - 68	Balakrishnam et al. (2012)
	The Bushehr Ports and marina in Persian Gulf	<6.80 – 35.4	Salch et al. (2016)
	Seto Inland Sea 2013 - 2014	682– 1,112	Kaonga et al. (2016)
	the marina and in fishing and commercial harbors in Panama	<0.08 – 2.8	Batista-Andrade et al. (2018)
	Tanabe Bay	8.2 – 9.3	Harino and Yamato (2021)
	Harima Nada Sea	33-128	This study
	Osaka Bay	43-83	This study
M1	Tanabe Bay	<0.1	Harino and Yamato (2021)
	Harima Nada Sea	60-128	This study
	Osaka Bay	104-377	This study
Dichlofluanid	Tanabe Bay	<0.1	Harino and Yamato (2021)
	Harima Nada Sea	67-8,038	This study
	Osaka Bay	104-263	This study
Chlorothalonil	Tanabe Bay	<0.1-8.2	Harino and Yamato (2021)
	Harima Nada Sea	31-2,975	This study
	Osaka Bay	49-165	This study