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Impact of Antifouling Biocide on Marine Environment

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Abstract

The impact of antifouling biocides on the marine environment in the northern part of Hiroshima Bay is the focus of this research. In this report, the effect on the marine environment was evaluated by the fertilized oyster egg's development. Unlike previous research that investigated Ot compounds Ot alternative antifoulants were chosen in this research. The Ot alternative antifoulants Diuron, Sea-Nine 211, TPBP, Irgarol 1051 and the latter's degradation product, M1, and Ot compounds were investigated.

At first the antifouling biocide's concentration in sea water was investigated, then the toxicity was tested, followed by the risk factor being calculated from the concentration and toxicity, and finally the environmental risk was evaluated. Sea-Nine 211 was present in the concentration that affected oyster embryo development in the northern part of Hiroshima Bay. So, it was clear that antifouling biocides had had a negative impact on the northern part of Hiroshima Bay's ecosystem.

Introduction

Organotin (Ot) compounds had been used for many years on ships, marine structures and fishery nets until detection of toxicity and accumulation characteristics. Ot compounds were subsequently prohibited on the 17th of September, 2008. Alternative compounds began to be used for ships with the thought that Ot alternative antifoulants would not accumulate in seawater and sediment as much as Ot compounds, due to the faster resolution speed. However, Ot alternative antifoulants have been detected at higher levels than initially expected which has caused concern about the possible effects on marine organisms.

In this research, sea water samples from the northern part of Hiroshima Bay were used to evaluate the toxicity of Diuron, Sea-Nine 211, TPBP, TPBOA and Irgarol 1051 on oysters. These chemicals were chosen due to their popular use in Japan following the prohibition in 1997 of paints that used TBT. For comparative reasons, TBT and TPT were also investigated. Oysters were chosen because of their high sensitivity to chemical compounds (Fichet et al. 1998; Wikfors et al. 1994; Stebbing et al. 1980) and the fact that they can be found worldwide.

The research sought to not only analyze the chemical compounds in survey points but also to measure the effect of antifouling biocides on fertilized oyster egg's development. Finally, the environmental risk was evaluated.

Material and Methods

Residual concentration of antifouling biocide in seawater samples

Survey point and sampling

A map of the sampling stations that were investigated is shown in Fig. 1. These include one marina, four fishery harbours, three environmental standard points and one dock around the Otagawa River mouth.

Points A and B are fishery harbours where a lot more leisure boats than fishing boats were anchored. At point C in the marina, only leisure boats were anchored. Point E was a fishery harbour which had about the same ratio of fishing boats to leisure boats. The point I fishery harbour had a high traffic of fishing boats but the number of boats which were anchored was low. The environmental standard point D was located offshore from the shipyard and the environmental standard point F was located offshore from the car factory. The environmental standard point G was located in a recess of Hiroshima Bay. Point H was located in the entrance to the dock and it was the point where the concentration of the organotin compounds had been high previously.

One liter of surface seawater and one liter of water from the sea floor (about 50cm from the sea floor) were taken from Hiroshima Bay. For the water sample from the surface seawater, a plastic bucket was used. For the water sample from the sea floor, a Vandone water sampler was used. In 2002 and 2004, the research was conducted in spring, summer, autumn and winter. However, in 2003, the research only took place in summer and winter.

Chemicals

Diuron was purchased from Tokyo Kasei Industries. Irgarol 1051 was obtained from Chiba Specialty Chemicals K.K. Sea-nine211 was supplied by Rhomans Hass. TPBP was donated by Hokko Chemical Industry. M1 was donated by Pf. Okamura in 1000ppm acetonitrile solution.

Pesticide grade acetone was purchased from Wako Pure Chemical Industries, Ltd. (Japan). Acetonitrile (HPLC grade) and pyridine (special grade) were obtained from Kanto Chemical Co., Inc. (Japan). The artificial seawater was made by dissolving the Daigo artificial seawater SP in Milli-Q water (1l). The Daigo artificial seawater SP was purchased from Nihon

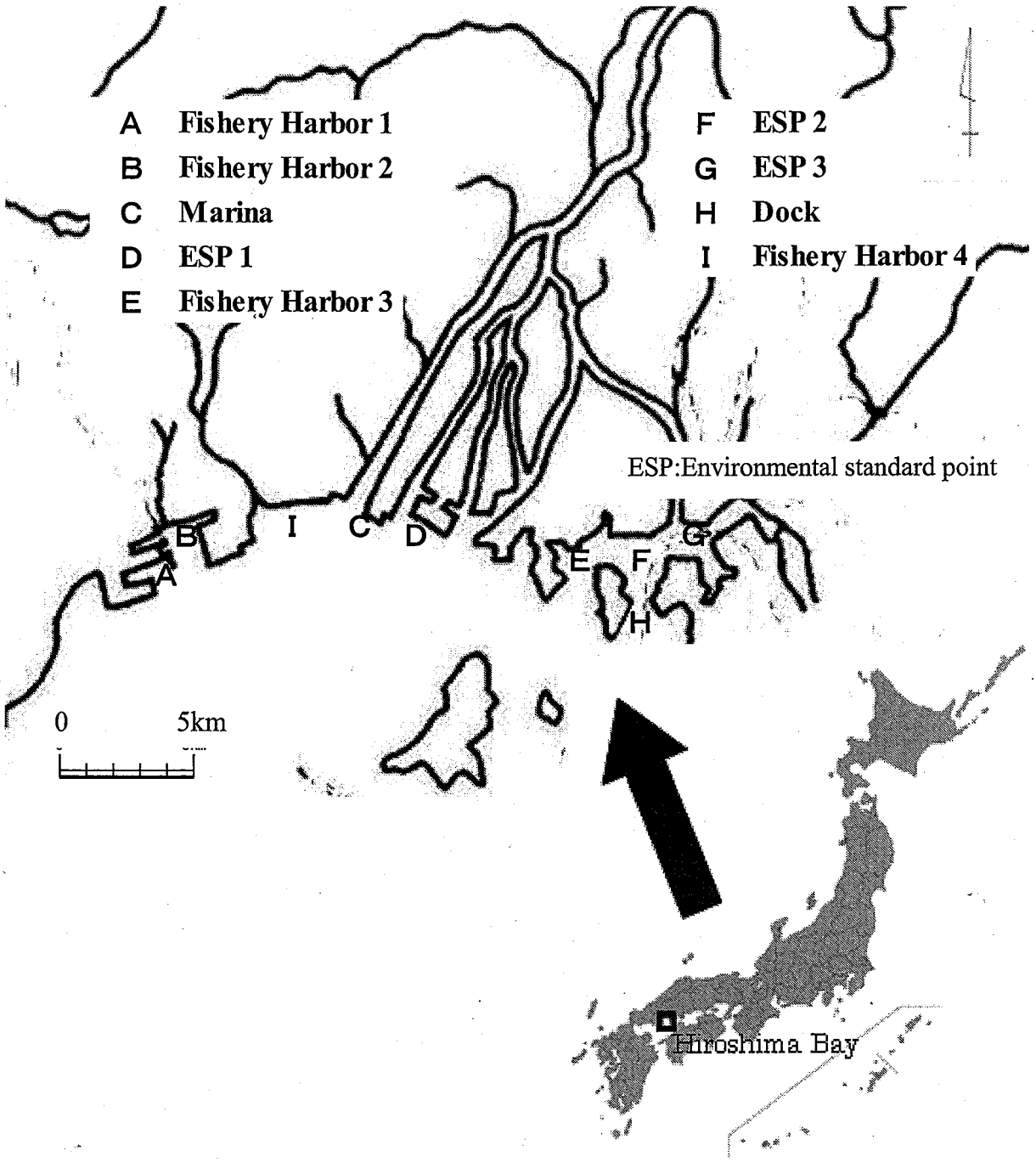

Fig. 1: Survey locations

Seiyaku Kogyo Co., Ltd. (Japan). The Milli-Q water that was made by the Gradient A10 (Millipore corp.) was used. Internal standard Phenanthrene-d10 and Fluoranthene-d10 (D10, 98% grade) which were obtained from Cambridge Isotope Laboratories Inc. (Andover, USA) and Atradine-d5 (pesticide grade) which was supplied by Dr. Ehrenstorter GmbH (Augsburg, Germany), were also used.

Devices and method

The Irgarol 1051 and M1 were analyzed by the JEOC AUTOMASS-SUN200. The analysis of Sea-nine211 and Diuron was conducted using the HITACHI ELITE La Chrome. TPBP analysis was done with the SHIMAZU LC-10AD.The detailed operation can be found in Tsunemasa et al. (2006, 2008)

Investigated materials

The investigated materials were Diuron, Sea-Nine 211, TPBP, Irgarol 1051 and the latter's degradation product M1 which were all thought to be popularly used in Japan.

Effect of antifouling biocide on oyster embryo development

Reagents and materials

The alternative Ot antifoulants Irgarol 1051, Diuron, Sea-Nine 211, Triphenylborane pyridine (TPBP) and triphenylborane octadecylamine (TPBOA) were used for the toxicity tests in this study, and TBT and TPT were used to compare these alternative Ot antifoulants with Ot compounds. Diphenylborane hydroxide (DPB), phenylborane dihydroxide (MPB), biphenyl, pyridine, phenol, benzene, and boric acid were used as the degradation products from TPBP or TPBOA (Okamura et al. 2009). Irgarol 1051 was obtained from Chiba Specialty Chemicals K. K. (Japan). Diuron, TBT and TPT were purchased from Tokyo Kasei Industries Ltd. Sea-Nine 211 (95%) was obtained from the Rhom and Hass Company (Philadelphia, USA). The TPBP, DPB, and MPB were donated by Hokko Chemical Industry (Japan). The TPBOA was donated by Benny-Toyama (Japan). The phenol, benzene, biphenyl (pesticide grade), and pyridine (spectroscopy grade) were purchased from Wako Pure Chemical Industries (Japan). The boric acid was obtained from Nakarai Chemical K.K. (Japan). Dilute stock solutions (1000 mg/l) were prepared by dissolving the standard materials in dimethyl sulfoxide (DMSO). The standard solutions were formed by diluting these solutions with artificial seawater which was prepared by diluting Daigo's artificial seawater SP purchased from Nihon Seiyaku Kogyo (Japan). The dimethyl sulfoxide (for biochemistry) and 10% formalin solution (for tissue fixation) were purchased from Wako Pure Chemical Industries (Japan). An alkaline formalin solution was prepared by further diluting these solutions with the artificial seawater stock. The oysters came from the breakwater in Itsukaichi Nishi Ward, Hiroshima, Japan. Professor Kenji Torigoe, from the Department of Education at Hiroshima University, identified the oysters used in the experiments as Crassostrea gigas.

Equipment

An Olympus CK40 biological microscope with a magnification of 100 times was used to photograph the oyster eggs, and the Motic Images Plus 2.2S image editing software package was used to count the number of oyster eggs.

Oyster toxicity tests

After initial trial and error tests, the procedure performed at the Fisheries Experimental Station in Hiroshima Prefecture, which simulates the conditions in a nursery was chosen. The detailed operation can be found in Tsunemasa and Okamura (2011).

After the experiments were concluded, the 10% lethal concentration (LC_{10}) and the 50% lethal concentration (LC_{50}) values were calculated using the Ecotox-Statics software program developed by the Japanese Society of Environmental Toxicology.

Results

Residual concentration of antifouling biocide in seawater samples

In this study, Sea-Nine 211, Diuron, Irgarol 1051 and the latter's degradation product M1 were detected in seawater from Hiroshima Bay whereas TPBP was not detected. Analysis results over the research period are shown in Table 1.

Comparison with detected values globally

In this research, Irgarol 1051 was only detected at 0.092μg/l at point H. Irgarol 1051 had been detected globally in seawater samples from the French Riviera in Monaco, Blackwater Estuary in the UK and East Anglia in the UK; at 14 - 640ng/l (Tolosa et al., 1996), 150 - 680ng/l (Voulvoulis et al., 2000) and 1 - 1,332ng/l (Lambert et al., 2006) respectively. These data were much higher than this research.

In this research, the maximum value of M1 detected was 1.3μg/l and in some cases it was undetected. M1 had been detected in seawater samples from Catalona in Spain, Southampton in the UK and East Anglia in the UK; at not detected (ND) - 4,000ng/l (Martinez et al., 2000), 1 - 59ng/l (Thomas et al., 2001), under 1 - 139ng/l (Lambert et al., 2006) respectively. With the exception of Spain, these figures were much lower than in this research.

The maximum value of Diuron detected in this research was 0.73μg/l and in some cases it was undetected. Diuron had been detected in seawater samples of the Humber River in the UK, Catalona in Spain and Southampton in the UK; at 40 - 8,700ng/l (House et al., 1997), up to - 2,000ng/l (Martinez et al., 2000), ND - 6,742ng/l (Thomas et al., 2001) respectively. These data were generally much higher.

This research showed the maximum value of Sea-Nine 211 detected as 0.10μg/l and undetected in some cases. In seawater samples of Catalona in Spain, Greece and Korsor harbor in Denmark it was detected; at ND - 3,000ng/l (Martinez et al., 2000), ND - 49ng/l (Sakkas et al., 2002), 30 - 72ng/l (Steen et al., 2004) respectively. This research's data was consistent with these data except for in Spain. The detailed results can be found in Tsunemasa et al. (2006).

Table 1: Minimum-Maximum concentration of antifouling biocides in sea water from Hiroshima Bay

Survey point	Irgarol 1051	M1	Diuron	Sea-Nine 211
A	ND	ND - 1.1	ND - 0.43	ND - 0.10
B	ND	ND - 1.3	ND - 0.73	ND - 0.10
C	ND	ND - 1.1	ND - 0.21	ND - 0.097
D	ND	ND - 0.17	ND - 0.17	ND
E	ND	ND - 0.10	ND - 0.24	ND - 0.067
F	ND	ND - 0.10	ND - 0.10	ND - 0.035
G	ND	ND	ND - 0.14	ND - 0.085
H	ND - 0.092	ND - 0.12	ND - 0.18	ND - 0.069
I	ND	ND - 0.060	ND - 0.17	ND - 0.052

ND: not detected (μg/L)

Effect of antifouling biocide on oyster embryo development

The alternative Ot antifoulants (Irgarol 1051, Diuron, Sea-Nine 211,TPBP and TPBOA) and the degradation products from TPBP and TPBOA (e.g., DPB, MPB, biphenyl, phenol, pyridine, benzene and boric acid) and Ot compounds (TBT and TPT), at concentrations ranging from 0.1 to 1000ug/ l, were used in the toxicity test on the fertilized oyster eggs. Photographs of the oyster embryo development after 2h and 24h were taken. Using these images, the toxicity of antifouling biocides was evaluated by examining cell division at 2h after fertilization and checking embryology (i.e., for D-shaped embryos) at 24h after fertilization.

Acute effects

This report investigated the survival rate of the eggs and the occurrence of deformity in D-shaped embryos. Survival rate and deformity rates of the oyster embryo after a period of 24h were shown in Fig. 2. In the report by His et al. (1997), four types of deformity were shown: convex hinge, indented shell margin, incomplete shell, and protruding mantle. In this research, only protruding-mantle deformities were observed.

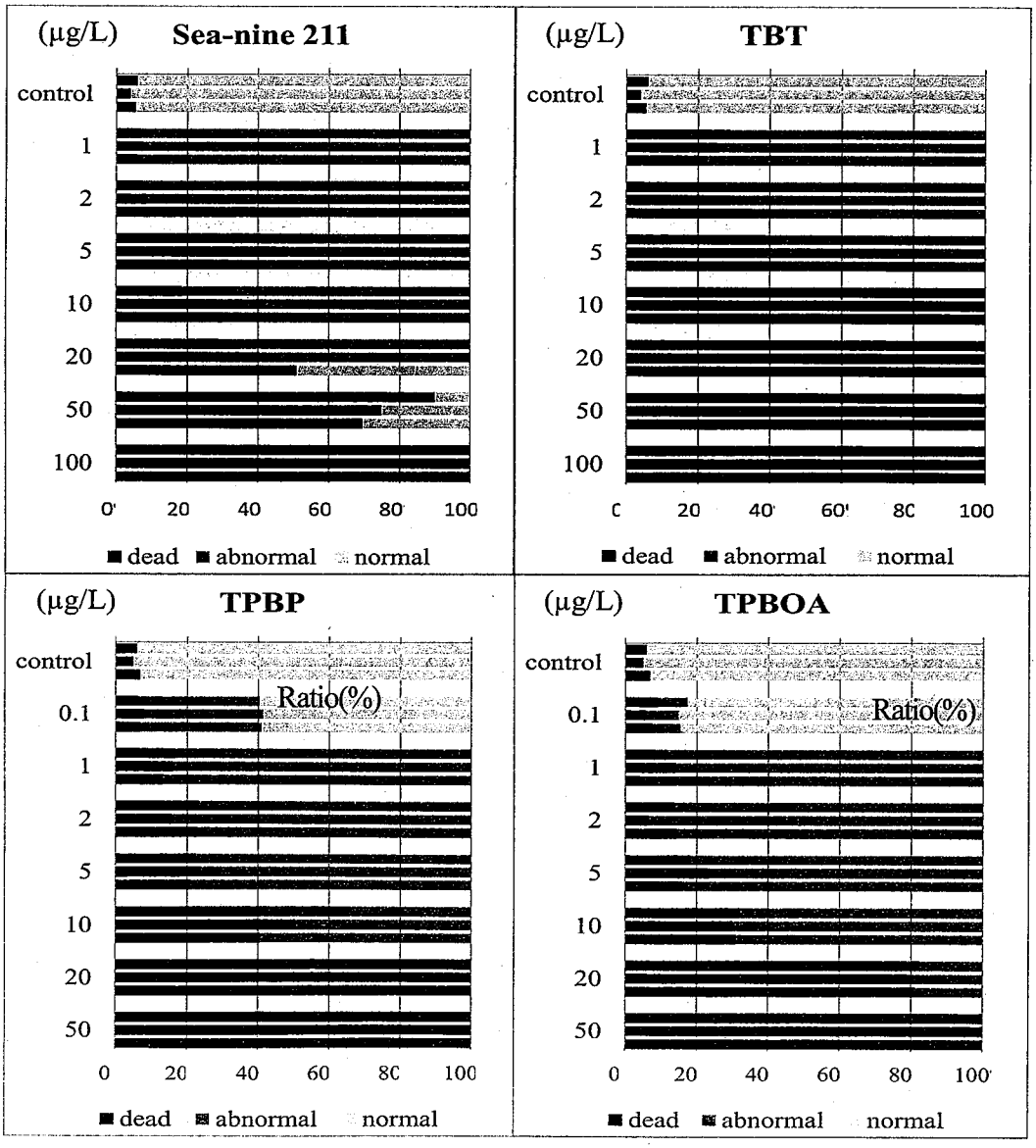

Fig. 2: Survival and deformity rates in a period of 24h

In Irgarol 1051, Diuron and the degradation products from TPBP and TPBOA samples, the oyster eggs developed favorably after a period of 2 and 24 h, and any evidence of any influence on the development of the eggs was not found, even at the maximum concentration (1000 ug/l). The above results are for the minimum concentration studied (0.1 ug/l) after a period of 24 h.

The results showed that in the Sea-Nine211 sample almost all the oyster eggs survived and became D-shaped embryos. Most D-shaped embryos developed naturally, but just over 10% developed protruding mantle deformity. About 10% of the eggs died in the TBT samples after cell division with just over 10% of the D-shaped embryos developing protruding mantle deformity. As for the TPT samples, almost all the eggs survived and became a D-shaped embryo. Around 15% of the D-shaped embryos developed protruding mantle deformity. As for TPBP, almost all of the oyster eggs survived. Approximately 70% of eggs became D-shaped embryos while the other 30% of the eggs showed signs of delayed development. Most D-shaped embryos were unaffected, but slightly less than 10% developed protruding mantle deformity. In the TPBOA samples, most of the eggs survived. All the surviving eggs became D-shaped embryos. Most D-shaped embryos were normal with slightly less than 10% of the D-shaped embryos developing protruding mantle deformity.

LC_{10} and LC_{50} values

The LC_{10} and LC_{50} values of each compound were calculated from the survival rate of the fertilized oyster eggs after an exposure time of 2 and 24 h using the Ecotox-Statics software package. The results are shown in Table 2. It can be seen that the toxicity of Irgarol 1051, Diuron and the degradation products of TPBP and TPBOA was not influential on the fertilized oyster eggs at high concentrations (1000ug/l). In the case of the other compounds, after a period of 24 h, the LC_{10} values of Sea-Nine 211, TBT, TPT, TPBP and TPBOA were 0.90, 0.36, 0.52, 0.58 and 2.2ug/l, respectively. At a period of 24 h, the LC_{50} values of these compounds were 17, 3.9, 3.7, 6.3 and 10ug/l, respectively. The detailed results can be found in Tsunemasa and Okamura (2011) and Tsunemasa et al. (2013).

Discussion

The alternative antifoulants 24 h LC_{10} (EC_{10}) analytical data was compared with that from other marine organisms that had been previously reported. The toxicity of Sea-Nine 211 and Irgarol 1051 towards *Mytilus edulis*, *Paracentrotus lividus*, and *Ciona intestinalis* were 7.1 and 800ug/l, 5.9 and 2900ug/l, and 5.8 and 930ug/l, respectively (Bellas 2006). Those results showed the same tendency as the results from this research, which was that Sea-Nine 211's toxicity was high and the toxicity of Irgarol 1051 was low. In other experiments, the No Observed Effect Concentration (NOEC) of Sea-Nine 211 towards *Paracentrotus lividus* was found to be 6.5ug/l (Bellas 2008). The NOEC of Sea-Nine 211 and Irgarol 1051 towards the green alga *Scenedesmus vacuolatus* (Arrhenius et al. 2006) and fucoid alga *Fucus serratus* (Braithwaite and Fletcher 2005) was 27 and 0.51ug/l, and 8.0 and 8.0ug/l, respectively. These results showed a different tendency from this research, in that the toxicity of Sea-Nine 211 was high, while the toxicity of Irgarol 1051 was low. Therefore, it is thought that Sea-Nine 211's toxicity is high in animal cells and is low in botanical cells.

Table 2: Lethal effects of antifouling compounds to oyster embryo

	2h		24h	
	LC_{10}	LC_{50}	LC_{10}	LC_{50}
TBT	2.6 (2.5-2.7)	16 (14-18)	0.36 (0.31-0.39)	3.9 (3.3-4.5)
TPT	2.4 (2.3-2.5)	14 (12-15)	0.52 (0.48-0.54)	3.7 (3.2-4.2)
Sea-Nine 211	7.4 (6.8-7.7)	28 (26-31)	0.90 (0.89-0.91)	17 (14-21)
TPBP	1.1 (1.0-1.1)	7.5 (6.7-8.5)	0.58 (0.55-0.60)	6.3 (5.4-7.4)
TPBOA	2.7 (2.6-2.8)	23 (20-26)	2.2 (2.1-2.8)	10 (9.5-12)
Diuron	>1000	>1000	>1000	>1000
Irgarol 1051	>1000	>1000	>1000	>1000
Degradation product				
DPB	>1000	>1000	>1000	>1000
MPB	>1000	>1000	>1000	>1000
Biphenyl	>1000	>1000	>1000	>1000
Phenol	>1000	>1000	>1000	>1000
Pyridine	>1000	>1000	>1000	>1000
Benzene	>1000	>1000	>1000	>1000
Boric acid	>1000	>1000	>1000	>1000

LC_{50} : 50% lethal concentration (μg/l) ( ) : 95% confidence interval
LC_{10} : 10% lethal concentration (μg/l)

According to the results of our laboratory tests, the effects of antifouling biocides on oysters are shown as follows (Tsunemasa and Okamura, 2011; Tsunemasa et al., 2013).
In case of the LC_{50}, the biocides order is as follows:
TBT = TPT = TPBP > TPBOA>Sea-Nine 211 >> Diuron = Irgarol 1051
In case of the LC_{10}, the biocides order is as follows:
TBT = TPT = TPBP > Sea-Nine 211 >TPBOA >> Diuron = Irgarol 1051
In both cases, TPBP's toxicity is the same as the toxicity of TBT and TPT. TBT, TPT and TPBP's toxicity is higher than that of Sea-Nine 211.

Environmental risk

In the evaluation of the environmental impact of these chemicals, a risk factor that used the predicted environmental concentration (PEC) divided by predicted no-effect concentration (PNEC) was used. When this value exceeded a risk factor of 1.0, it was decided the chemical had an influence on the environment (Hampel et al. 2007). The PNEC on the aquatic organism was calculated from the examination result of the acute or chronic effect in the organism species divided by assessment factor. In this research, the chemicals resolved in the toxicity experiment were considered, so the assessment factor was set at 10.

TBT, TPT, TPBP and TPBOA were not detected in the sea water samples from previous research. Therefore, they did not indicate environmental risk. In the case of Sea-Nine 211, the toxicity towards the oyster eggs was highest of the four chemicals detected in this research. The predicted environmental concentration in Barcelona, Osaka and Hiroshima, was 3.3ug/l (Martinez et al. 2000), 0.02ug/l (Harino et al. 2005) and 0.1ug/l (Tsunemasa et al., 2006) respectively. The deformity rate of oyster embryo exceeded 10% in the solution with a concentration of 0.1ug/l, so the NOEC was determined to be 0.1ug/l. PNEC which is the NOEC divided by assessment factor was 0.01ug/l and the chemicals risk factor divided by their PNEC values, was 330, 2 and 10 respectively. In all cases, the risk factor was much higher than a value of 1.0, and so it indicates that Sea-Nine 211 influences the marine environment.

In this research period, Sea-Nine 211 was detected at values that exceeded Sea-Nine 211's PNEC in almost all survey points. This means that Sea-nine 211 is present in concentrations that effect oyster embryo's development in the northern part of Hiroshima Bay. If the effects that were observed in the laboratory took place in nature, at least 10% of oyster embryos would exhibit a deformity. It is reasonable to say that oyster embryos which show signs of deformity are unlikely to develop into adult oyster.

In recent times, the indication of delayed development of oysters in Hiroshima Bay has become more noticeable. If this trend continues, oyster numbers will decrease. If the oyster's numbers decrease, there are a number of influences that would be possible. Oysters play an important role as natural filters for the environment so a decline in numbers could see an adverse affect on water quality of Hiroshima Bay. Also, various marine life (e.g. *Acanthopagrus schlegelii, Takifugu poecilonotus*) in Hiroshima Bay use oysters as a food source so any change in numbers could affect not only the organisms which feed off them but also alternative food sources. It is clear that any decline in oyster numbers would have a negative impact on the northern part of Hiroshima Bay's ecosystem.

As mentioned previously, Sea-Nine 211, Diuron and Irgarol 1051 were detected while TPBP was not detected in the northern part of Hiroshima Bay. Essentially, the best antifouling biocides should show a high toxicity around the hull, but when they are released into the sea water they should resolve quickly so they do not affect the organisms which inhabit the sea water. Sea-Nine 211 has high toxicity and when it is released into the sea water it remains for a long time so affects organisms. TPBP's toxicity is the same as the toxicity of TBT and TPT, but resolves quickly in the sea water, so it does not affect organisms as much. If the toxicity is almost the same, the material (e.g. TPBP) with the highest resolution speed should be used to lessen the impact on the ecosystem. For this reason, TPBP is the best antifouling biocide from the investigated antifouling biocides.

Conclusions

・Sea-Nine 211 should be substituted with another compound because although it is present in lower concentration than other chemical substances, it is the most harmful to oyster embryos of the investigated compounds.

・Chemical substances bring negative impacts to the northern part of Hiroshima Bay's ecosystem.

・The antifouling biocides should show a high toxicity around the hull, but quickly resolve in the sea water.

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