The Influence of Organotin Alternative Antifoulants on Oyster Embryology

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1.Introduction

Organotin(Ot) compounds that were used for many years as antifouling biocides have become a problem because of their toxicity and accumulation characteristics. In September 2008, Ot compounds were prohibited from being used worldwide. From 1997 in Japan, the production of paints that contain TBT compounds was prohibited, and so alternatives to Ot antifoulants have been used since then. We have evaluated the toxicity of alternative Ot antifoulants (i.e.,Diuron, Irgarol 1051, and Sea-Nine211) and Ot compounds (TBT and TPT) using oysters that inhabit a large area of Hiroshima Bay.

2.Materials and Methods

2.1 Reagents and Materials

We used the alternative Ot antifoulants Irgarol 1051, Diuron and Sea-Nine211 for the toxicity tests, and we used TBT and TPT to compare these alternative Ot antifoulants with Ot compounds. We made dilute solutions (1000mg/l) by dissolving the standard materials in dimethyl sulfoxide (DMSO) and then made standard solutions (1.0-1000μg/l) by diluting the dilute solutions with artificial seawater.

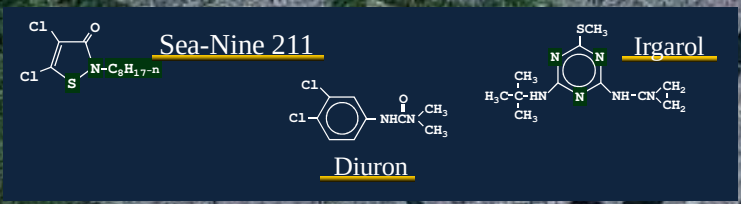

Fig.1Structers of the alternative Ot compounds

2.2 The oyster toxicity test

We placed a volume of 1ml of standard solution into a 24hole-microplate.We prepared three wells with the same concentration.

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We added a mature egg to each well, along with a volume of 25μl of artificial seawater.

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We diluted the sperm, which was preserved in a refrigerator, with artificial seawater by1000 times, added a volume of 25μl of artificial seawater to each well, and then used it to fertilized the sample.

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We used a constant temperature tank, maintained at 25℃, for the microplates during cultivation. After a period of 2h and 24 h, we observed each well under the microscope and indentified the development stages of 200 oyster eggs.

3.Result and Discussion

The condition of the oysters is shown in Fig.2.

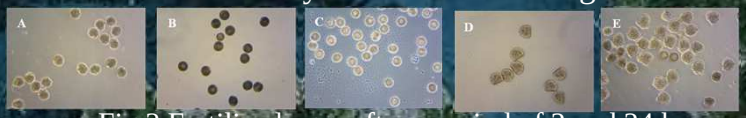

Fig.2 Fertilized eggs after a period of 2 and 24 h.
(A):Oyster eggs with normal cell division(Sea-Nine211,1ppb,2h); (B):Oyster eggs that didn't survive(TBT,100ppb,2h); (C):Oyster eggs with no development(Sea-Nine211,100ppb,24h);(D)Normal D-shaped embryos (control,24h); and (E) Deformed D-shaped embryos (TBT,5ppb,24h).

3.1 LC10,LC50

We calculated the LC10 and LC50 values of each compound from the survival rate of the fertilized oyster eggs after an exposure time of 2 and 24 h using the data from the Ecotox-Statics software package. The results are shown in Table1.

Table 1 Lethal effects of antifouling compounds on oyster embryos

	2h		24h	
	LC10	LC50	LC10	LC50
TBT	2.7(2.6-2.9)	14(13-16)	0.69(0.64-0.70)	3.3(2.9-3.7)
TPT	4.1(3.9-4.3)	13(12-14)	1.4(1.4-1.5)	2.5(2.4-2.7)
Sea-Nine 211	4.3(4.0-4.5)	27(24-30)	1.3(1.2-1.3)	13(11-15)
Diuron	>1000	>1000	>1000	>1000
Irgarol 1051	>1000	>1000	>1000	>1000

LC50：50 % lethal concentration (μg/L)　　(　) :95 % confidence interval
LC10：10 % lethal concentration (μg/L)

3.2 Influence on deformity

We have looked at the number of living and dead eggs after a fertilization period of 24h.

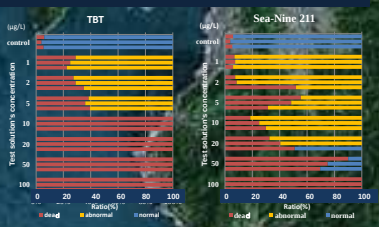

Fig.3 The survival rate and deformity rate after a period of 24h.

Figure 3 shows the survival and deformity rates after a development period of 24h.

4. Conclusions

We evaluated the toxicity of alternative Ot antifoulants using the oyster's embryology. The results showed that the toxicity of Diuron and Irgarol 1051 was very low, and the toxicity of Sea-Nine 211 was almost the same as that of TPT.