EFFECT OF PYRIDINE TRIPHENYL BORANE(KH101) ON THE REPRODUCTION OF CALANOID COPEPOD, ACARTIA AMORII BRADFORD

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ABSTRACT

Currently documented that pyridine triphenyl borane (KH101) is possible to use as an alternative of tributyltin based antifouling biocide in coastal and marine environment. But its impact on marine life especially on smaller zooplankton is so far unknown. The present study was conducted to determine the effect of KH101 on adult survival, egg production and egg hatch of a calanoid copepod, *Acartia omorii* under bath administration. Survival was observed every 24h for 10 days. Each 10 individuals of adult female were incubated at different concentrations of KH101 (0.001, 0.01, 0.1, 1, 10 and 100μg/L) with food (*Isochrysis* sp.+*Tetraselmis* sp., ratio 1:1). Egg production was examined through the incubation of single gravid female with above conditions but only at 0.001, 0.01, 0.1 and 1μg/L of KH101 concentrations. Egg hatching success was determined for each 60 eggs at different concentrations of KH101, which were obtained from pre-exposed female at the same concentration. LC_{50}s were obtained in 0.1, 1, 10 and 100μg/L concentrations of KH101 after 168, 96, 48 and 24h, respectively. Significant (P<0.05) decrease in egg production and egg hatching success were obtained at 1μg/L level. Result suggests that persistent level above 0.1μg/L of KH101 affects the reproduction success of the calanoid copepod, *A. omorii*.

Keywords: copepod, KH101, survival, egg production, egg hatch

INTRODUCTION

After a long debate since 1970s regarding on the use of organotin biocide in antifouling coatings, established a global legal ban on the use of TBT in antifouling hull coatings. For that needed an alternative antifouling compound, which as possible 'comparative' replacement of TBT. Already a few number of new alternative antifouling compounds have been identified and recommended to use as an antifouling biocide for hull coating (Yonehara *et al*., 2000; Voulvoulis *et al*., 2002). Pyridine triphenyl borane (KH101) is one of them and generally used in antifouling purpose for fishing net and has been proposed to use as an antifouling biocide for hull coatings (Yamada and Kakuno, 2003). It is the safe and effective antifouling biocide, which also recommended by the Japanese shipbuilding association to successfully prevent the fouling of submerged surface in the sea (Yonehara *et al*., 2000).

Very limited work has been conducted on the effect of KH101 on aquatic organisms. Kobayashi and Okamura (2002) reported that up to 10fg/L has no effect on the egg development of sea

urchin (*Hemicentrotus pulcherrimus* and *Anthocidaris crassispina*). Furthermore, Okamura *et al*. (2002b) observed 50% lethal concentration (LC_{50}) at 0.084mg/L after 14 days exposure period in juvenile rainbow trout *Oncorhynchus mykiss* and also mentioned that it is more toxic to animal than as herbicide. No study found on the effect of KH101 on the crustaceans reproduction especially on zooplankton.

In zooplankton community, copepods are as much as 70% of them and one of the main primary consumers in marine food pyramid (Steele, 1970). Therefore, antifouling chemicals could crucially damage to coastal ecosystems if they adversely affect copepod production. The calanoid copepod, *Acartia* sp., is the most abundant and important copepod in Japanese coastal waters (Yamaji, 1956) and also in other international water areas (Wilson, 1932) where pollutant materials could be easily accumulated. In this paper, the most common calanoid copepod in coastal water, *Acartia omorii* were used to determine the ecotoxicity of KH101 by: (i) acute toxicity for adult female survival, (ii) determining the egg production capabilities of exposed animals subjected to lethal and sub lethal concentrations and (iii) egg hatching success in different concentration of KH101 exposed eggs (i.e. eggs were collected from the same concentration of exposed animal in the same condition).

MATERIALS AND METHODS

Culture of *Acartia omorii*

Test animal *A. omorii* was collected from an estuarine pond of Tokyo University of Marine Science and Technology beside the Keihin canal by selective (200μm) plankton netting. After collection a single gravid female was immediately sorted and put into an egg collection tank (Fig.1), which consisted of acrylic tube with plastic mesh (200μm) at the bottom and outer glass jar fitted with tube. Eggs, deposited at the bottom of outer jar, were collected and reared in 5L glass jar for mass monoculture (ca. 30-40 adult individuals/liter) and held in temperature-controlled chamber.

Water and food management

Seawater stored at 5°C was filtered through Whatman GF/F glass fibre filter and diluted with ultra pure water (Milli-Q) to obtain salinity of 25‰ and used for culture. In mass culture, water was exchanged twice per week (after 3-day 50% and after 6-day 80% of total volume). In egg production and survival observations, water was exchanged 2/3 of total volume at every 24h interval. *Tetraselmis* sp.+ *Isochrysis* sp. (1:1 in cell density) were applied as a quantity of ca. 2×10^4 cells/ml. In mass culture practice, the ratio of food was applied twice a week (after water exchange), while in survival and egg production observations every 24 hour interval.

Test substance

The test substance, KH101 was obtained from Hokko Chemicals Ltd., Japan (molecular weight 321, melting point at 210°C) was dissolved in dimethyl sulphoxide (DMSO, 99.5%) at a concentration of 5,000mg/L. All exposure media were prepared before 12h of application with filtered seawater, stored in Pyrex media bottles and preserved in an incubator (20°C) at dark

condition during the study. All types of glassware and egg tanks were dipped in detergent solution ('CLEAN 99', 20ml/L, Clean Chemical Co., Japan) for 24h and washed with water. They were then dipped in 0.1N HCL for 24h, washed with water and finally rinsed with deionized water.

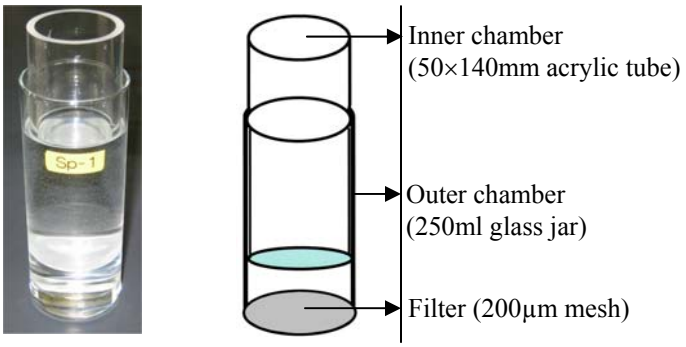

Figure 1. Photograph and diagram of an egg collection tank.

Experimental conditions

Overall conditions were same in all observations including mass culture system. Temperature was 20±1°C and salinity 25‰, which were ambient conditions at the time of sample collection. Light intensity was 80-90μ$Em^{-2}S^{-1}$ with photocycle of 12L:12D and the exposure pattern was bath administration.

Survival observation

Adult copepods were incubated by the use of 50ml glass test-tube (length 150mm, diameter 25mm). Here 30ml exposure media was added to each test-tube (working volume 30ml). Ten adult (♀) copepods were allocated to each treatment for the period of 10 days. The exposure concentrations were applied as 0.001, 0.01, 0.1, 1, 10 and 100μg/L levels. In every 24h interval, a 20ml exposure media was exchanged with newly prepared media and food was also applied. Survival was checked at 24h interval using a microscope. Death of copepod was determined when they could not show any movement for 60 seconds.

Egg production observation

Randomly picked up gravid female from mass culture system was selected for egg production observations, which consist of 10-replications per treatment for the period of 10 days. In each replication one female was incubated in 250ml egg tank containing 150ml exposure media. Eggs were collected in every 24h interval and the exposure concentrations were applied only at 0.001, 0.01, 0.1 and 1μg/L of KH101 because of high mortality at 10 and 100μg/L concentration. All eggs were settled in the bottom of the outer chamber through the mesh. In the collection procedure, an egg tank with existing 50ml exposure medium was shifted to a new outer chamber with 100ml of new medium containing the same concentration of KH101. Eggs in old outer chamber with 100ml of rest medium were collected by a pipette and counted under a microscope.

Egg hatching observation

Randomly picked up 10 gravid females from the laboratory mass monoculture stock were incubated in the same condition with egg production observation. 10 eggs were randomly collected from the laid of those 10 females at 72-96h exposure periods and incubated in 6-well chambered micro plate containing 10ml exposure medium in each chamber. Six replications were conducted in each treatment. The observation period and exposure concentration were same as egg production observation and hatched eggs were counted under a microscope at every 24h interval.

Statistical analysis

In all observation, we first carried out control, solvent control and KH101 exposure treatments, which were considered as different treatment groups. Because the data from the control and the solvent control did not differ significantly in any of the measurement variables, we treated these two groups into a single group for analysis, which will be referred to as control (C) group. The data on egg production and egg hatching success in all treatment were performed using SISTAT software (SISTAT 8.0, 1998) and were analyzed by one-way ANOVA followed by Post hoc comparison with Fisher's Least-Significant-Difference (LSD) test and the significance was ascertained at $P<0.05$.

RESULTS AND DISCUSSION

Adult survival

The survival was observed 100% after 48h of exposure period up to 0.1μg/L concentration. All individuals were died at 100 and 10μg/L concentrations after 24h and 48h exposure periods,

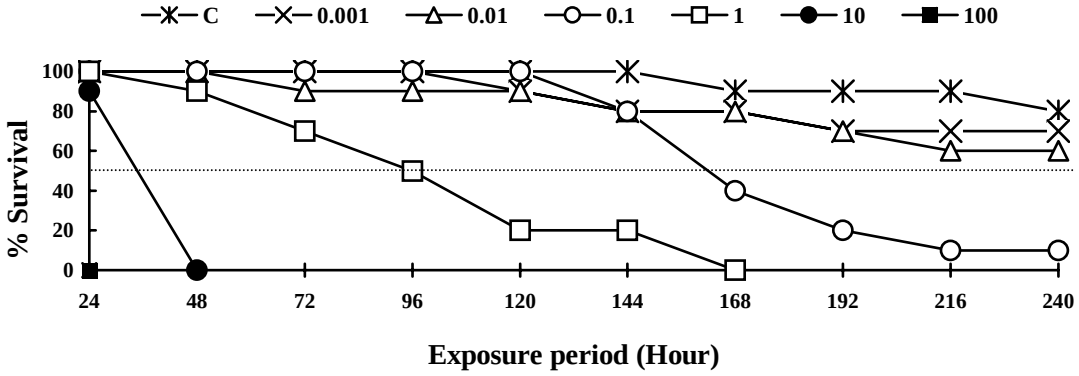

Figure 2. Total survival (%) of adult copepod (♀) at different time interval.

respectively. In lower concentrations, LC_{50}s were obtained at 1 and 0.1μg/L concentrations after 96 and 144h of exposure periods, respectively (Fig.2). More than 60% were survived up to 0.01μg/L concentration at the end of 240h exposure periods. All individuals of control treatment were alive up to 144h of exposure periods and 80% survived at the end of observation in this treatment. So, increasing level of KH101 has harmful effect on adult survival. Okamura *et al*.

(2002b) observed LC_{50} at 61µg/L after 21 days exposure period in juvenile rainbow trout *Oncorhynchus mykiss*. Therefore, calanoid copepod is more sensitive to KH101 at very low concentration of <0.1µg/L. In comparison with TBT (100% mortality at 3µg/L after 48h bath administration), KH101 shows lower toxicity to this species (Hossain *et al*., 2003) but comparatively more toxic than alternative biocide Irgarol 1051, which observed LC_{50} after 192h exposure at 100µg/L concentration (Hossain *et al*., 2004b). In control, 100% were survived up to 144h and at the end of observation period only 20% were died. It should be considered as normal because all individual were picked up from mass culture system, where several age groups were present. In this study, adult individuals (♀) were selected through the superficial viewing with the help of microscope and all individuals might not be in same age group.

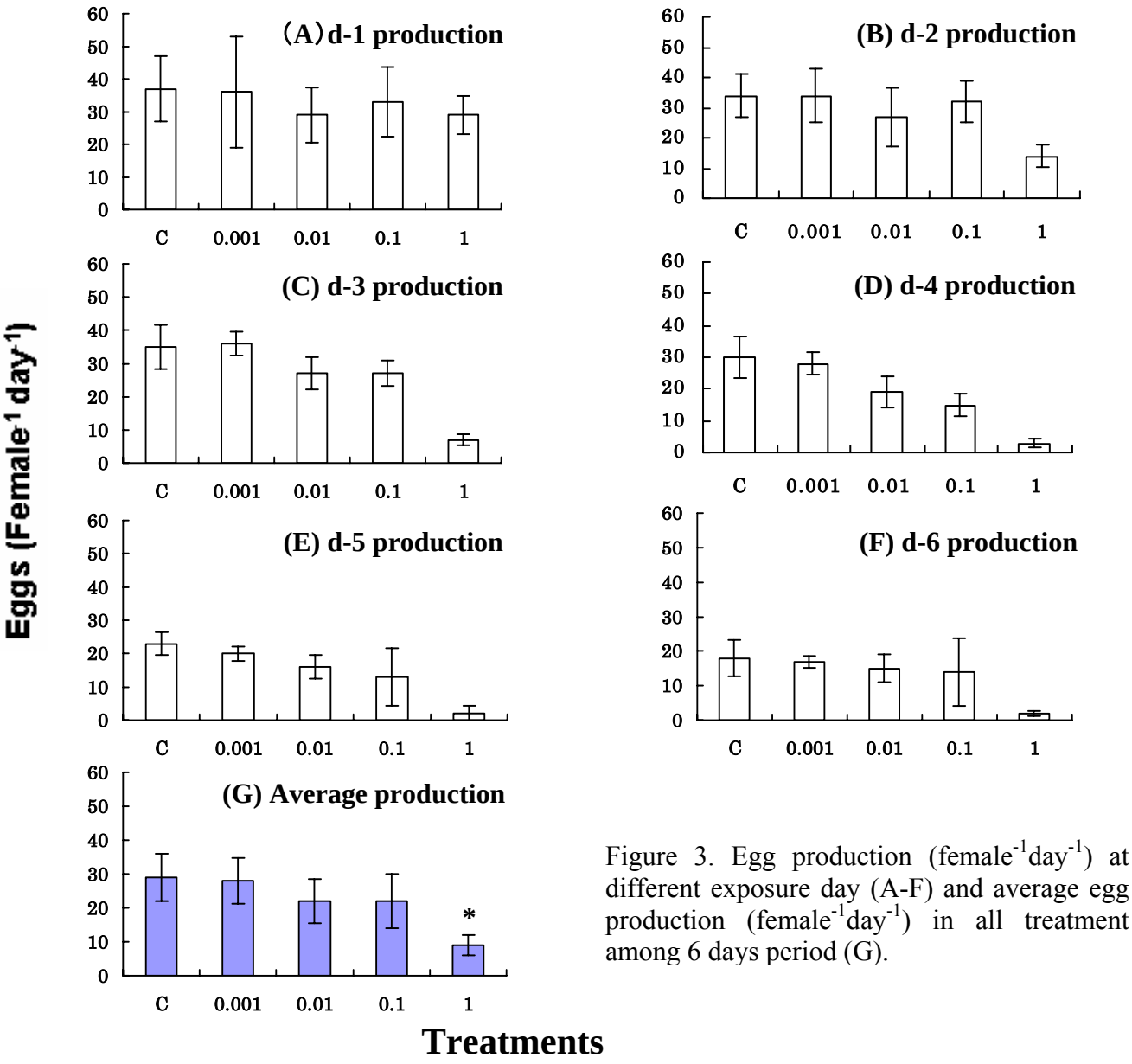

Figure 3. Egg production (female^{-1}day^{-1}) at different exposure day (A-F) and average egg production (female^{-1}day^{-1}) in all treatment among 6 days period (G).

Egg production

Almost equal egg productions (female^{-1}day^{-1}) were observed up to 0.1μg/L concentrations but significant decrease at 1μg/L concentration (Fig.3). These results indicate that up to 0.1μg/L concentration of KH101 has not any effect on the egg production of calanoid copepod. Egg production observation was conducted for 10 days but after 7 days all individuals were died at 1μg/L treatment. Therefore, statistical analysis was conducted for the data obtained during initial 6 days. In comparison with TBT (significantly decrease the egg production at 0.01μg/L concentration under bath administration), it has less effect on the egg production of calanoid copepod (Hossain *et al.*, 2003). However, with other largely used alternative biocide Irgarol 1051 (significantly decrease the egg production at 100μg/L concentration), it has more adverse effect on egg production of calanoid copepod (Hossain, 2004b). In control treatment, mean egg production per individual was 18-37eggs (female^{-1}day^{-1}). In another study, Hossain *et al*. (2004b) reported 15-43 egg production in the same condition of *A. omorii*. So, normal egg production result was obtained in this observation. Though in this study, biochemical analysis of animal body was not conducted, it is considered that the response of KH101 was absent in all treatments up to 0.1μg/L exposure concentration. Therefore, our results demonstrate that, KH101 impacts on reproduction activities in *A. omorii* at 1μg/L concentration and it is warrant acceptance of the hypothesis regarding the reproductive effect of KH101 on copepod.

Egg hatch

Hatching rate was significantly decreased at 1μg/L concentration but almost same level up to 0.1μg/L concentrations (Fig.4).

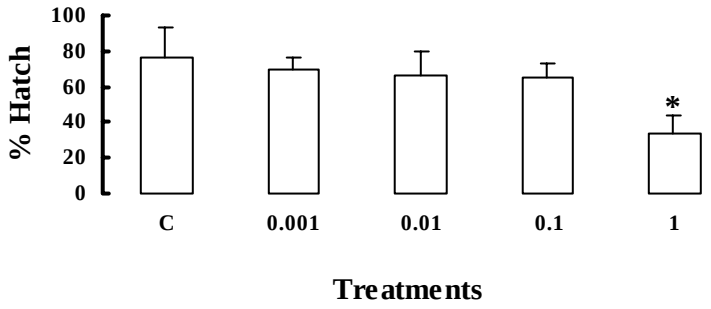

Figure 4. Average egg hatch (%) in each treatment after 10 days of exposure observation.

So far, no information was found about the effect of KH101 on hatching success of marine zooplankton eggs. Hypothesis was being considered that, any exposure to breeders also effects on their newly produced eggs. Therefore, observation was performed to determine any relationship of KH101 to egg hatching success of calanoid copepod eggs. In this study, we observed 100ng/L concentration of KH101 does not give any effect on the hatch of *A. omorii* eggs and 1μg/L concentration significantly decrease the egg hatching success. So, KH101 strongly affect on the hatching success of calanoid copepod eggs and the possible consideration is the toxicity stress may affect on the egg viability of newly produced eggs. Lindley *et al*. (1999) reported that exposure of organochlorine compounds (pentachlorophenol and

dichlorobenzene) to female copepod *Acartia bifilosa* also affected on their newly produced egg's viability as well as egg hatching success. Generally healthy female exhibits good reproductive quality. Therefore, the observed decrease in hatching success under exposure treatment probably resulted not from the effect on egg's itself but from the effect on the condition of female while eggs were produced. The effect of KH101 on calanoid copepod egg hatch is much lower than that of organotin biocide TBT (significantly decreased egg hatch at 0.1µg/L), which observed in *A. omorii* under same condition (Hossain, 2003). However, in comparison with Irgarol 1051 (no effect observed on egg hatch up to 100µg/L), it has serious effect on egg hatch of calanoid copepod (Hossain, 2004b).

In control treatment, average hatching rate was observed 76.7% after 10 days incubation period. Ueda (1981) also found that seawater isolated *A. omorii* (*clausi*) eggs hatched up to 78% and hatched almost synchronously around 23hrs after spawning. Uye (1980) reported that about 70 to 98% of *A. omorii* (*clausi*) eggs hatched at 20°C. Hossain *et al*. (2003) reported that the egg hatching success at 20°C was 81% after 10 days incubation period in *A. omorii*. Therefore, compared to the other investigations on the egg hatch of *Acartia* sp., it is agreed that good hatching success was found in this observation. So, it is conclude that KH101 affect on the egg hatching success of calanoid copepod at 1µg/L concentration under bath administration.

CONCLUSION

Significantly different effect of KH101 was ascertained in egg production and egg hatch at 1µg/L concentration of bath exposure but distinct harmful effect was observed in adult female survival at 0.1µg/L and above level. So it's concluded that alternative biocide KH101 has sever effect on the reproduction success of calanoid copepod *Acartia omorii*, at the persistent level of 0.1µg/L in the coastal and marine environment. However, this effect is much lower than that of TBT.

Acknowledgements

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