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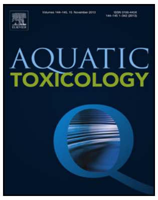

Acute and chronic toxicities of zinc pyrithione alone and in combination with copper to the marine copepod *Tigriopus japonicus*

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ABSTRACT

Zinc pyrithione (ZnPT) is a widely used booster biocide in combination with copper (Cu) in antifouling paints as a substitute for tributyltin. The co-occurrence of ZnPT and Cu in coastal marine environments is therefore very common, and may pose a higher risk to marine organisms if they can result in synergistic toxicity. This study comprehensively investigated the combined toxicity of ZnPT and Cu, on the marine copepod *Tigriopus japonicus*, for the first time, based on both 96-h acute toxicity tests using adult copepods and chronic full-life cycle tests (21 d) using nauplii <24-h old. As ZnPT has been reported to be easily trans-chelated to copper pyrithione (CuPT) in the presence of Cu, the acute toxicities of CuPT alone and in combination with Cu on adult copepods were also assessed. Our results showed that ZnPT and Cu exhibited a strong synergistic toxic effect on the copepod in both acute and chronic tests. During the acute test, the mortalities of adult copepods increased dramatically even with an addition of Cu at concentrations as low as 1–2 μg/L compared with those exposed to ZnPT alone. Severe chronic toxicities were further observed in the copepods exposed to ZnPT–Cu mixtures, including a significant increase of naupliar mortality, postponing of development from naupliar to copepodid and from copepodid to adult stage, and a significant decrease of intrinsic population growth when compared with those of copepods exposed to ZnPT or Cu alone. Such synergistic effects might be partly attributable to the formation of CuPT by the trans-chelation of ZnPT and Cu, because CuPT was found to be more toxic than ZnPT based on the acute toxicity results. Mixtures of CuPT and Cu also led to synergistic toxic effects to the copepod, in particular at high Cu concentrations. A novel non-parametric response surface model was applied and it proved to be a powerful method for analysing and predicting the acute binary mixture toxicities of the booster biocides (i.e., ZnPT and CuPT) and Cu on the copepod. To better protect precious marine resources, it is necessary to revise and tighten existing water quality criteria for biocides, such as ZnPT and CuPT, to account for their synergistic effects with Cu at environmentally realistic levels.

1. Introduction

Tributyltin (TBT) has restricted application on ship hulls since the early 1980s due to its severe impacts to marine life, and a global complete ban of TBT in antifouling paints has entered into force from 2008 (IMO, 2001; Yebra et al., 2004; Fent, 2006). A new generation of booster biocides such as zinc pyrithione (ZnPT), copper pyrithione (CuPT), Irgarol 1051, diuron, and Sea-Nine 211, has been increasingly used as TBT substitutes globally, and they are usually applied in combination with Cu in which the percentage of booster biocides is usually around 5%, while the percentage of Cu can be up to 40% or more (Lassen et al., 2001; Yebra et al., 2004; HK AFCD, 2008). Elevated concentrations of many of these biocides (e.g., Irgarol, diuron, Sea-Nine 211) and their degradation products have been detected worldwide in coastal areas such as marinas and harbours as a consequence of their increased use (Konstantinou and Albanis, 2004). There is an increasing concern over the environmental occurrence, fate, toxicity and persistence of these biocides (Evans et al., 2000; Konstantinou and Albanis, 2004). Though progresses have been made on aforementioned aspects (Thomas, 2009), most relevant studies are focused on single biocides, and there is still a lack of data on the mixture toxicities of these biocides (Howell and Evans, 2009).

Another main concern is that dramatically increased application of Cu-based antifouling biocides has led to elevated Cu concentrations in coastal waters and sediments around the world (Schiff et al., 2007), particularly in coastal areas with busy

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harbours. The mean dissolved Cu concentration was reported to be 8.8 μg/L (range: <0.5–75 μg/L) in the Essex/Suffolk estuaries in UK between 1992 and 1996 (Matthiessen et al., 1999), 8.5 μg/L in marinas of the San Diego region, USA (Schiff et al., 2007), and 11.23 μg/L (range: 1.61–48.52 μg/L) in beaches of Acapulco, Mexico (Jonathan et al., 2011). Along the coastal waters in Perth, Western Australia, Department of Water (2009) found that the total Cu concentration in water ranged from <1 to a maximum of 12,000 μg/L, and exceeded the ANZECC guideline (1.3 μg/L) in six out of eight study sites, while Cu concentration in sediment ranged from 1.5 to 17,900 μg/g dry weight (dw), and exceeded the ANZECC guideline (65 μg/g dw) in three out of the eight study sites. Likewise, sediment Cu concentration was found to range from 5.8 to 846 μg/g dw with a median concentration at 60.6 μg/g dw in the Toulon Bay, France (Tessier et al., 2011), range from 256 to 279 μg/g dw in three out of the four study sites in eastern Aegean coastal waters, East Mediterranean (Onen et al., 2011), and range from 47 to 286 μg/g dw with a mean concentration at 117 μg/g dw in Sydney estuary, Australia (Birch et al., 2014).

As both booster biocides and Cu are the main components of most of the Cu-based antifouling products, their co-existence in the marine environment is expected to be not unusual especially in marinas and harbours with high boating activity and low water exchange rate. The booster biocides and Cu may interact with each other, leading to additional, synergistic or antagonistic combined toxic effect to marine organisms (Teisseire et al., 1999), and understanding these interactions is necessary to determine the ultimate environmental impacts of the booster biocides on marine ecosystems (Hertzberg and MacDonell, 2002). For example, mixtures of Irgarol 1051 and Cu have been shown to have an additive toxicity to a marine copepod (Bao et al., 2013). However, there is a paucity of data on the combined toxicity of other commonly used booster biocides (e.g. ZnPT) and Cu to marine species, and almost all relevant documented studies are merely based on acute tests (Mochida et al., 2006; Zhou et al., 2006; Koutsaftis and Aoyama, 2007; Bao et al., 2008).

ZnPT and CuPT are both metal pyrithiones, and were first introduced into the market as antifouling booster biocides in 1990s. ZnPT-based antifouling paints have been intensively applied worldwide, especially in Europe and South Korea (Thomas, 1999), and in Japan, where ZnPT and CuPT are the two most frequently used biocides in antifouling products (Okamura and Mieno, 2006). ZnPT can easily transchelate with Cu into CuPT; a partial transchelation of ZnPT into CuPT was detected in seawater with naturally present Cu, and a total transchelation of ZnPT into CuPT was detected when ZnPT was released from Cu-based antifouling paints (Grunnett and Dahllöf, 2005). Thus, the environmental fate and persistence of ZnPT are closely related to those of CuPT in the marine environment. Both ZnPT and CuPT were found to be highly toxic to aquatic autotrophic species (e.g. microalgae and cyanobacteria) and animals (Turley et al., 2000; Bao et al., 2011, 2012). However, up to now there are relatively few data on the toxicity of both metal pyrithiones to aquatic organisms.

ZnPT and CuPT were marked as environmentally neutral and non-persistent in the aquatic environment as they can undergo fast photodegradation to form less toxic compounds in seawater under direct sunlight (half-life <2 min; Turley et al., 2000, 2005; Bellas, 2005). However, both metal pyrithiones are suggested to be persistent in marine environments where light is limited such as waters and sediments shaded under parked vessels in marinas and harbours, or in water columns with high turbidity; and ZnPT may accumulate in the sediment as CuPT and manganese pyrithione (MnPT) (Galvin et al., 1998; Maraldo and Dahllöf, 2004). A field experiment reported a half-life of 209 min at a depth of 0.5 m for CuPT, and photodegradation was absent at 1 m or more below surface after 1-h exposure to penetrating sunlight (Grunnett and Dahllöf, 2005). Marcheselli et al. (2010b) also found that half of the initial ZnPT quantity remained after 48-h light exposure (indirect sunlight plus laboratory fluorescent lamps). Notably, CuPT had been detected in marine sediment samples from Vietnam (range: <2–420 μg/kg-dry wt.) and Japan (range: 8–22 μg/kg-dry wt.) (Harino et al., 2006, 2007). A total pyrithione concentration of 105 nM was detected in water samples collected from Mersey estuary, UK indicating the possible existence of pyrithiones like ZnPT and CuPT in marine water columns (Mackie et al., 2004). Marcheselli et al. (2010b) reported a basal level of ZnPT and ionized pyrithione (PT^-) in the mussels *Mytilus galloprovincialis* inside a busy harbour of Italy, indicating that the level of ZnPT in the harbour is high enough to induce a detectable accumulation in native organisms, and the study also showed a rapid accumulation of ZnPT in the mussel in laboratory experiment, suggesting that ZnPT has potentials to accumulate in the trophic chain.

Tigriopus japonicus (Copepoda, Harpactidae) is a common intertidal rocky shore species that has a wide geographical distribution, mainly found along the Western Pacific coast. *T. japonicus* was selected for this study as it is ecologically important, highly abundant and small in size; while it has a short generation time and high fecundity, it can be easily cultured under laboratory conditions (Raisuddin et al., 2007). It is also a good candidate for chronic toxicity tests (e.g., life cycle test) due to its distinct post-embryonic developmental stages (6 naupliar stages, 5 copepodid stages and an adult stage), dimorphic sex and high fecundity of females with multiple broods of eggs developed sequentially after a single mating event (Itô, 1970; Raisuddin et al., 2007; Kwok et al., 2009). Additionally, toxicity tests conducted with this copepod had shown a good reproducibility (Kwok et al., 2008).

In order to investigate the combined toxicity of ZnPT and Cu, this study investigated both the acute (with mortality as the endpoint) and chronic (life cycle test with larval mortality, developmental time and intrinsic rate of increase as endpoints) toxicities of ZnPT alone and in combination with Cu on the marine copepod *T. japonicas*. The acute toxicities of CuPT alone and in combination of Cu on the adult copepod were also examined to elucidate the combined toxicity of ZnPT and Cu. Response surface models have proven to be successful to fit all experimental data in a single model and clearly visualizing all combined toxicity data in a three dimensional concentration response surface (Gessner, 1995; Bao et al., 2008, 2013). We applied and compared three different response surface approaches, namely the Loewe parametric response surface (CARS; Greco et al., 1995), response additive response surface (RARS; Bao et al., 2013), and the non-parametric response surface (NPRS; Gessner, 1995; Greco et al., 1995) models to describe and predict the combined acute toxicity of binary mixtures of individual biocides and Cu.

2. Materials and methods

Standard 96-h semi-static acute aquatic toxicity tests of ZnPT and Cu alone on adult copepods *T. japonicus* were first conducted in order to determine the median lethal concentration (LC50) for each biocide (please refer to the details of the test conditions in Section 2.3). Suitable ZnPT and Cu concentrations were chosen according to their LC50 values and pilot study results for the binary mixture acute toxicity test of the two biocides on the adult copepods, and for the binary mixture chronic toxicity test of the two biocides using newly hatched copepod nauplii less than 24-h old (Table 1). In order to investigate whether the combined toxicity of ZnPT and Cu was simply due to the formation of CuPT by the transchelation of ZnPT and Cu, the acute toxicities of CuPT alone and in combination with Cu to the adult copepod were also investigated (Table 1). For a better comparison of the toxicities between the