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Toxicity of antifouling biocides to the intertidal harpacticoid copepod *Tigriopus japonicus* (Crustacea, Copepoda): Effects of temperature and salinity

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Abstract

Intertidal harpacticoid copepods are commonly used in eco-toxicity tests worldwide. They predominately live in mid-high shore rock pools and often experience a wide range of temperature and salinity fluctuation. Most eco-toxicity tests are conducted at fixed temperature and salinity and thus the influence of these environmental factors on chemical toxicity is largely unknown. This study investigated the combined effect of temperature and salinity on the acute toxicity of the copepod *Tigriopus japonicus* against two common biocides, copper (Cu) and tributyltin (TBT) using a $2 \times 3 \times 4$ factorial design (i.e. two temperatures: 25 and 35 °C; three salinities: 15.0‰, 34.5‰ and 45.0‰; three levels of the biocide plus a control). Copper sulphate and tributyltin chloride were used as the test chemicals while distilled water and acetone were utilised as solvents for Cu and TBT respectively. 96h-LC50s of Cu and TBT were 1024 and 0.149 $\mu g l^{-1}$ respectively (at 25 °C; 34.5‰) and, based on these results, nominal biocide concentrations of LC0 (i.e. control), LC30, LC50 and LC70 were employed. Analysis of Covariance using 'concentration' as the covariate and both 'temperature' and 'salinity' as fixed factors, showed a significant interaction between temperature and salinity effects for Cu, mortality increasing with temperature but decreasing with elevated salinity. A similar result was revealed for TBT. Both temperature and salinity are, therefore, important factors affecting the results of acute eco-toxicity tests using these marine copepods. We recommend that such eco-toxicity tests should be conducted at a range of environmentally realistic temperature/salinity regimes, as this will enhance the sensitivity of the test and improve the safety margin in line with the precautionary principle.

Keywords: Hong Kong; Lethal concentration; Antifouling; Metals; Organotins

1. Introduction

Marine pollution resulting from antifouling biocides in Hong Kong has long been a serious problem. There are two major reasons: (1) heavy boat traffic—Hong Kong is one of the busiest passenger and cargo ports in the world while also possessing an active fishing industry; (2) regular maintenance such as hull cleaning and repainting of local and visiting vessels at local shipyards (Ko et al., 1995). Hong Kong also has a considerable mariculture industry, which often uses antifouling agents for their boats and cages thereby also contributing to such pollution. All these warrant a high consumption of antifouling paints locally. Commonly used active ingredients of antifouling paints in Hong Kong include tributyltin (TBT) and copper (Cu) compounds. It was estimated in 1998 that the average annual consumption of TBT based antifouling paint at the dockyards in Hong Kong was as high as 232,500 l (EPD, 1998).

Tributyltin was marketed as the perfect biocide and has been used extensively since 1970s until regulations were introduced to control sale and usage of TBT in

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recent years (Champ and Seligman, 1996). Tributyltin is so effective as a biocide that at part-per-trillion levels it is already toxic to marine organisms (Leung et al., 2001). Unfortunately the toxicity of TBT is non-specific, and it has a high bioaccumulation factor and persistence in sediments that pose a great problem to the marine environment (Ko et al., 1995). Environmental impacts of TBT were first discovered after the collapse of French oyster farming industry in 1975 due to increased TBT usage (Alzieu et al., 1986). Adverse effects of TBT on marine organisms including shell malformation in oysters (Alzieu et al., 1986), mortality of mussel larvae (Beaumont and Budd, 1984) and imposex in gastropods (Bryan and Gibbs, 1991) were well established. As a result, the International Maritime Organization (IMO, 2002) has stated "the global instrument should ensure a global prohibition on the application of organotin compounds which act as biocides in antifouling systems on ships by 1 January 2003, and a complete prohibition on the presence of organotin compounds which act as biocides in antifouling systems on ships by 1 January 2008". It is, however, arguable that toxic substances like DDT are still being produced and used in some developing countries nowadays, although they have been banned in developed countries since 1970. Likewise, it is anticipated that organotin compounds, as effective biocides, will be continuously produced and used in the near future, especially in developing countries and countries that do not join the IMO.

Previous studies have suggested that contamination with TBT in the marine environment of Hong Kong is serious and with little evidence of improvement or recovery (Lau, 1991; Ko et al., 1995; Cheung et al., 2003; Li, 2003), even after a partial ban in 1992. Marine sediments in Hong Kong contain a wide range of concentrations from 2.4 to 2837 $mg\,kg^{-1}$ (dry weight) of TBT with the highest TBT level of 2837 $mg\,kg^{-1}$ found in sediment collected from Tsing Yi near the shipyards involved in the stripping of TBT-painted vessels (Cheung et al., 2003).

Copper has been used as an antifoulant since the time of the Phoenicians (Champ and Seligman, 1996). It is an essential metal that is necessary for some enzymes, protein structure (e.g. haemocyanin in marine invertebrates) and iron utilisation in marine organisms (Gross et al., 2003), but at elevated levels it is especially toxic to invertebrates. It can also lead to growth inhibition and reproduction disturbances in marine invertebrates (Nimmo and Hamaker, 1982). Copper contamination in Hong Kong waters is common and widespread; Cu concentrations in marine sediment in relatively unpolluted Eastern Hong Kong waters were about 20 $mg\,kg^{-1}$ (EPD, 2002). However, in highly urbanized areas such as Victoria Harbour, Cu concentrations in the sediment have been found to reach a maximum of 4000 $mg\,kg^{-1}$ (EPD, 2002). The toxicity of Cu is mainly from the bioavailable fraction and has been shown to be dependent on the concentration of the free copper ion (i.e. Cu^{2+}) (Sunda and Guillard, 1976). This relationship is supported by the description of positive relationship between free Cu^{2+} concentration and growth inhibition in 38 clones of marine phytoplankton (Brand et al., 1986). Physicochemical characteristics of Cu ions in seawater are influenced by myriad environmental features such as pH, temperature, salinity, redox potential and dissolved organic matter (Gledhill et al., 1997; Di Toro et al., 2001).

Conventional eco-toxicology experiments are carried out under standardized laboratory conditions where most environmental parameters are held constant. This is not a good analogue of the highly variable natural environment. As most saltwater eco-toxicity tests are conducted at fixed temperature and salinity, the influence of these environmental factors on the chemical toxicity is largely unknown. Various authors have demonstrated that toxicity of a chemical varies with both salinity and temperature. For example, Hall et al. (1995) documented that toxicity of cadmium to the calanoid copepod *Eurytemora affinis* and sheephead minnow *Cyprinodon variegatus* varies with salinities of the test medium. Sogorb et al. (1988) also showed that toxicity of fluvalinate on the red swamp crayfish *Procambarus clarkii* significantly increased with increased test temperatures. Nonetheless, the combined effect of temperature and salinity on the toxicity of biocides such as Cu and TBT to the common intertidal copepod species has yet to be revealed.

Intertidal harpacticoids copepods, such as *Tigriopus* sp. have been commonly used in eco-toxicity tests worldwide (O'Brien et al., 1988; Barka et al., 2001). These copepods are dominant species of high shore rock pools and are necessarily tolerant to wide ranges of temperatures and salinities. Due to its high abundance throughout the world, small size, short generation time, high fecundity and high culturability, *Tigriopus japonicus* is an ideal marine eco-toxicity test organism. Therefore, it is the aim of this experiment to investigate the combined effect of temperature and salinity on the acute toxicities of the two common biocides, Cu and TBT to *T. japonicus*.

2. Material and methods

2.1. Standard acute toxicity tests

All test animals were collected from high shore supralittoral pools at the Cape d'Aguilar Marine Reserve, Hong Kong. Two sets of standard 96-h static-renewal acute toxicity tests were conducted for copper (Cu: reagent grade $CuSO_4 \cdot 5H_2O$; molecular weight = 249.7 g) and tributyltin (TBT: reagent grade TBT-Cl; molecular weight = 325.5 g) to determine the 96h-LC30, LC50