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Embryotoxicity of the antifouling biocide zinc pyrithione to sea urchin (*Paracentrotus lividus*) and mussel (*Mytilus edulis*)

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Abstract

The effects of the new antifouling compound zinc pyrithione (Zpt) on the embryonic development of sea urchin (*Paracentrotus lividus*) and mussel (*Mytilus edulis*) were investigated in laboratory toxicity tests. The median effective concentrations (EC_{50}) were 7.7 nM for sea urchin embryos and 8 nM for mussel embryos. Toxic effects of Zpt on the larval growth of the sea urchin were detected at 0.5 nM. Predicted environmental concentrations of Zpt in pleasure craft harbours are higher than the predicted no effect concentrations for sea urchin and mussel embryos, indicating that Zpt may pose a threat to those species from exposure in the field.

Keywords: Zinc pyrithione; Antifouling; Biocide; Sea urchin; Mussel; Embryo-larval bioassay

1. Introduction

Since the international ban on the use of TBT in antifouling paints the biocide zinc pyrithione (Zpt) has been introduced into the market (Arch Chemicals Inc.) as an interesting replacement for traditional TBT-based antifouling paints (Voulvoulis et al., 2002; Maraldo and Dahllöf, 2004). Thus, according to Arch Chemicals reports, the continued strong demand for substitute biocides used in marine antifouling paints resulted in a sales increase in 2004 of 15% over 2003, and sales for 2005 are expected to increase by approximately 8 to 10% (http://www.archchemicals.com/Fed/Corporate/News). Although a great research effort has been carried out on the study of Zpt characteristics, little is known about its toxicity to marine organisms (Goka, 1999; Kobayashi and Okamura, 2002; Petersen et al., 2004). Therefore, studies on the effects of Zpt to marine organisms are urgently needed.

The bioassays with embryos of bivalves and echinoderms are widespread tools for the evaluation of toxicity of marine pollutants and their sensitivity allow their use in environmental risk assessment studies in coastal ecosystems (Carr and Chapman, 1995; His et al., 1999; Beiras et al., 2003). In view of recent studies revealing extreme toxicity (lowest observed effect concentration = 0.1 attoM) of Zpt to the sea urchin embryo-larval bioassay (Kobayashi and Okamura, 2002), we undertook the present work to evaluate the toxicity of Zpt to *Mytilus edulis* and *Paracentrotus lividus* embryonic development. Furthermore we investigated a sublethal response in early larval growth to be used in the assessment of marine pollution that has not previously been frequently used.

2. Materials and methods

2.1. Experimental solutions

Stock solutions were made up by dissolving analytical grade zinc pyrithione (Sigma–Aldrich Steinhem) in a

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non-toxic organic dissolvent, dimetylsulfoxide (DMSO) approximately 1 h before the beginning of the experiments. The experimental concentrations were obtained by diluting the stock solution in artificial seawater (ASW). The ASW was prepared as in Zaroogian et al. (1969) but salinity was adjusted to 34 ppt adding distilled water. All glassware was acid-washed (HNO_3 10% vol.) and rinsed with acetone and distilled water before the experiments.

2.2. Sea urchin

The method used in the *Paracentrotus lividus* test has been previously described by Fernández and Beiras (2001). Gametes were obtained by dissection from a single pair of adults. Four hundred fertilised eggs were delivered into 20 ml glass vials with Teflon-lined caps containing the experimental solutions.

2.3. Mussel

Mature *Mytilus edulis* were induced to spawn by thermal stimulation in separated beakers with 0.2 μm filtered seawater. Eggs from a single female were transferred to a 100 ml measuring cylinder and their quality was checked under microscope. Sperm solution was stored at 4 °C until use. Sperm mobility was checked under microscope and a few μl were added to the egg suspension and carefully stirred to allow fertilisation. Fertilised eggs (ca 30 eggs/ml) were transferred to glass vials with Teflon-lined caps containing the experimental solutions.

The vials were incubated at 20 °C (*P. lividus*) or 18 °C (*M. edulis*) for 48 h in the dark, to avoid photodegradation of the Zpt. After the incubation period larvae were preserved by adding a few drops of 40% buffered formalin and the percentage of fully developed 4-arm pluteus and D-veliger larvae ($n = 100$), and the mean larval length of the pluteus larvae ($n = 25$) were recorded. Five replicates per treatment, five ASW controls, and five DMSO controls were assayed for each experiment. The same amount of DMSO (less than 1 ml/l) was added to each treatment. The tests were repeated at least three times with different batches of embryos. Control embryogenesis success was always above 90% for sea urchin and above 80% for mussels.

2.4. Statistical analyses

The lowest observed effect concentrations (LOEC) were determined by ANOVA and Dunnetts test. The raw data were frequently found not to be normally distributed and were therefore arcsine-transformed to achieve normality (Hayes, 1991). The EC_{50}, defined here as the toxicant concentrations causing 50% reduction in the embryogenesis success, and their 95% confidence intervals (CI) were calculated according to the probit method. Previously to performing EC_{50} calculations, data were normalized to the control mean percentage of larval abnormality using Abbots formula (Emmens, 1948). Statistical tests were performed according to Newman (1995) and Sokal and Rohlf (1995).

3. Results and discussion

Zinc pyrithione has long been used as a bactericide and fungicide in agriculture and in antidandruff shampoos (Shuster, 1984; Marks et al., 1985; Voulvoulis et al., 1999) and has more recently been used as an alternative antifouling booster biocide to traditional coatings containing organotin compounds (Voulvoulis et al., 1999; Meseguer Yebra et al., 2004). However, limited research has been conducted to assess the toxicology of this compound and few studies have investigated

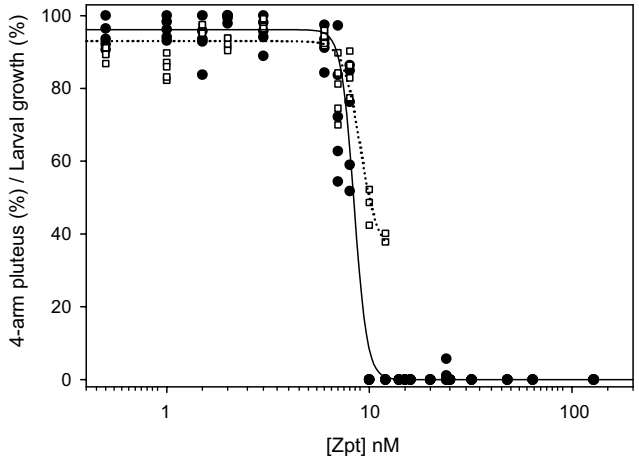

Fig. 1. Percentage of 4-arm pluteus larvae (filled symbols) and larval growth (open symbols) after 48 h exposure of *P. lividus* fertilised eggs to different concentrations (nM) of Zpt.

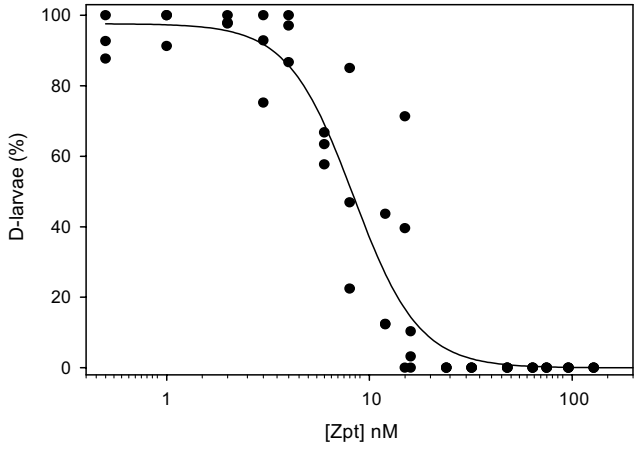

Fig. 2. Percentage of D-veliger larvae after 48 h exposure of *M. edulis* fertilised eggs to different concentrations (nM) of Zpt.