Marine Pollution Bulletin xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Binary mixture toxicities of triphenyltin with tributyltin or copper to five marine organisms: Implications on environmental risk assessment

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ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form 6 February 2017
Accepted 14 February 2017
Available online xxxx

Keywords:
Synergistic
Antagonistic
Additive
Concentration addition
Response addition

ABSTRACT

Triphenyltin (TPT) often coexists with tributyltin (TBT) and Cu in coastal waters worldwide. The combined toxic effect of TPT and TBT has always been assumed to be additive without any scientific proof, and the combined effect of Cu and TPT on marine organisms has not been vigorously studied. This study, therefore, investigated the acute toxicity of binary mixture of TPT/Cu and TPT/TBT to five selected marine species including *Thalassiosira pseudonana*, *Skeletonema costatum*, *Tigriopus japonicus*, *Brachionus koreanus* and *Oryzias melastigma*. The interaction between TPT and TBT or Cu was modeled antagonistic based on concentration addition (CA) model, while it was synergistic according to response addition (RA) model. Both model well predicted the toxicity of binary mixtures to the five organisms. As for the environmental risk assessment, CA overestimated the toxicity in most cases and thus is a more conservative model than RA model for assessing the toxicity of these chemical mixtures.

1. Introduction

Organotin compounds (OTs), primarily tributyltin (TBT) and triphenyltin (TPT), are effectual biocides that have been widely applied for various industrial and agricultural purposes. Among these applications, OT-based antifouling products used on hulls of vessels and submerged mariculture facilities are the main sources of OT pollution in the marine environment. Since the late 1980s, distribution of OTs in different marine compartments including water, sediment and biota has been widely documented in many countries (Gibbs et al., 1987; Horiguchi et al., 1994; Gomez-Ariza et al., 1998; Leung et al., 2006). In most of these published literatures or reports, TBT was the most abundant residue in different marine compartments while TPT ranked the second (Yi et al., 2012). In Hong Kong, however, the scenario is different. For example, TPT levels (0.5 to 2.2 ng L^{-1}) were close to those of TBT (<detection limit to 3.9 ng L^{-1}) in seawater samples collected from Marine Protected Areas of Hong Kong, while levels of TPT (20.9 to 48.2 ng g^{-1} dry weight) were much higher than TBT (1.7 to 8.7 ng g^{-1} dry weight) in sediment samples (Xu et al., 2016). According to an investigation on OTs' tissue burden of *Reishia clavigera* collected from Hong Kong coastal waters, TPT is the most predominant residue, accounting for up to 90% of the total OTs, and TBT is the second abundant residue (Ho and Leung, 2014). In spite of the difference in composition and proportion of OTs, TPT and TBT were found to coexist with each other in most cases.

To date, some studies have been conducted on the toxic mechanisms of TBT or TPT on aquatic organisms and many of them suggested a similar toxic mechanism of action shared by both compounds (Fent and Meier, 1994; WHO, 1999; Janer et al., 2005). For example, both TBT and TPT inhibit the CYP1A activity in fish hepatoma cells (Brüschweiler et al., 1996); in the echinoderm *Paracentrotus lividus*, both compounds can inhibit the activity of testosterone acyltransferase (Janer et al., 2005); and they can bind to retinoid X receptors in marine gastropod *R. clavigera* and thus play an important role in inducing imposex (Nishikawa et al., 2004). Generally, the combined toxic effect of TPT and TBT has been assumed to be additive, based on which the European Food Safety Authority (EFSA) established the tolerable daily intake value of this group of OTs to be 0.25 μg kg^{-1} bw d^{-1} (EFSA, 2004). There are, however, some exceptions to this consumption. In the common dog-whelk *Nucella lapillus*, TBT can induce imposex at concentrations as low as 0.5 ng L^{-1} while an injection of TPTCl doesn't even at a concentration as great as 1000 ng L^{-1} (Bryan et al., 1988). Santos et al. (2006) suggested a synergistic effect of TBT and TPT mixtures on the gastropod *Bolinus brandaris* having imposex promotion as endpoint. The above mentioned study, to the best of our knowledge, is the only one that have investigated the toxicity of different OT mixtures, though it simply had one concentration combination of TPT and TBT and only considered the case of response addition. Up to date, there is no relevant, specifically designed study to investigate and elucidate the interactions among different OTs.

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http://dx.doi.org/10.1016/j.marpolbul.2017.02.031
0025-326X/

Due to globally restricted use of OTs in antifouling systems, copper (Cu) based antifouling paints have been emerging since early 1990s and are now dominating the market (Bao et al., 2008). Given that Cu can be readily released from the antifouling system, the wide application of the copper-based antifouling coatings on ship hulls, submerged marine infrastructures and mariculture cages has led to elevated concentrations of Cu in coastal waters and sediments worldwide (Schiff et al., 2007). For example, the Cu level was as high as 20 μg L^{-1} in some busy ports in the United Kingdom (Matthiessen et al., 1999), and it reached about 160–170 mg kg^{-1} dry weight in sediment samples collected from Victoria Harbour, Hong Kong (EPD, 2011).

Copper often coexists with different biocides in coastal areas, especially those having heavy shipping activities. In mussel samples (*Mytilus* sp.) collected from harbours in France, the levels of OTs ranged from 0.04 to 2.2 μg g^{-1} dry weight, and Cu levels were 6.6 to 22.7 μg g^{-1} dry weight (Devier et al., 2005). The interactive effect between Cu and biocides has been recorded in many organisms. For example, Cu and zinc pyrithione have synergistic effect on the diatom *Thalassiosira pseudonana*, polychaete larvae *Hydroides elegans* and amphipod *Elasmopus rapax* (Bao et al., 2008); and synergistic effect between Cu and four antifouling biocides including Igorol 1051, dichlofluanid, tolylfluanid and Sea-Nine 211 were found in the sea urchin embryos of *Glyptocidaris crenularis* (Xu et al., 2010). On the contrary, antagonistic effects between copper and biocides were observed in two phytoplanktonic microorganisms, the green alga *Dunaliella tertiolecta* and the diatom *Navicula forcipata* (Gatidou and Thomaidis, 2007). Although OTs, especially TPT and TBT, always coexist together with Cu, the combined effect of OTs and Cu to marine organisms are still largely unknown.

As risk evaluation solely based on the toxicity of a single contaminant is insufficient to determine its eventual environmental impacts, understanding the interactions (i.e. additive, synergistic or antagonistic effect) between different contaminants is very important in ecological risk assessment (ERA) (Hertzberg and MacDonell, 2002). Concentration addition (CA) and response addition (RA) models are the most commonly used models to predict the joint toxicity of mixtures for similar- and dissimilar-acting toxicants, respectively. Developed from CA and RA model, two commonly used parametric response surface approaches, namely the Loewe parametric response surface (CARS) and the response additive response surface (RARS) model, are used to describe the effects of binary/multiple mixtures with a specific interacting parameter (α of CARS model and ρ of RARS model).

Given the above, this study was designed to (1) investigate the acute toxicity of binary mixture of TPT/TBT and TPT/Cu to five selected marine species including two diatoms *T. pseudonana* and *Skeletonema costatum*, the copepod *Tigriopus japonicus*, the rotifer *Brachionus koreanus* and the marine medaka *Oryzias melastigma*, covering three trophic levels; and (2) to apply and compare CARS and the RARS model to describe and predict the combined acute toxicity of both TPT/Cu and TPT/TBT mixtures.

2. Materials and methods

2.1. Chemical preparation and concentration selection

A range of stock solutions of TPTCl at concentrations ranging from 10^3 to 10^6 μg L^{-1} was made by dissolving TPTCl (>95%; Sigma, USA) in dimethyl sulfoxide (DMSO; ACS reagent, 99.9%; Sigma, USA). A stock solution of Cu at the concentration of 10^6 μg L^{-1} was prepared by dissolving Copper (II) sulphate pentahydrate ($CuSO_4$; 99.5%; BDH Chemicals Ltd. Pode, England) in distilled water. Working solutions at designated nominal concentrations were prepared by diluting stock solution in filtered artificial seawater (FAS; 32.5 ± 1 ppt or 15.0 ± 1 ppt; 0.45 μm, Millipore, USA) using volumetric flasks. The concentrations for toxicity test of binary mixtures were selected based on acute toxicity test of single chemicals for each organism, and five concentrations of each chemical (i.e. concentrations close to 0, 50% of EC10, EC10, EC50 and EC80) were finally designated. For both TPT/TBT and TPT/Cu mixtures, a total of 25 concentration combinations (5 × 5 factorial treatments) were applied.

Control (f/2-Si medium for the diatoms; FAS at 32.5 ± 1 ppt for the copepod and medaka fish; 15.0 ± 1 ppt FAS for the rotifer) and solvent control (control with 0.01% DMSO) were also carried out for all the selected organisms in parallel, and the solvent content in all treatments were adjusted to 0.01% DMSO except control.

2.2. Acute toxicity test for the two diatoms

An initial algal cell concentration of 10^5 cells mL^{-1} for both *T. pseudonana* (Strain code: CCMP 1015, CCMP, USA) and *S. costatum* (Strain code: CCMP 1335; CCMP, USA) were exposed to different concentrations of the test chemicals or their mixtures dissolved in 5 mL of f/2-Si medium in 10-mL glass vials. The exposure, which last 96 h, were conducted in an environmental chamber with the temperature of 25 ± 1 °C and the light cycle of 14 h light: 10 h dark. No water change was conducted during the exposure. All the vials were shaken twice every 24 h to re-suspend the precipitated cells. After the exposure, the number of cells was counted with a hemocytometer and a relative growth rate of each treatment was calculated.

2.3. Acute toxicity test for the rotifer

The rotifers used in this study were obtained from the Department of Molecular and Environmental Bioscience, Hanyang University, Korea, which was originally sampled from sampled at Uljin (36°58′43.01′′N, 129°24′28.40′′E) in South Korea. They were cultured in FASW (15‰, 0.45 μm filter membrane, 25 ± 1 °C, 12 h light: 12 h dark), and maintained with the marine microalgae *Tetraselmis suecica*. A 24-h exposure was conducted for the rotifers. In brief, 10 neonates of *B. koreanus* (<24 h old) were designated into 4 mL test solution ($n = 3$) in 12-well cell culture plates (BD Biosciences, UK). The exposure was static without water change or feeding. Mortality of the rotifers, which was defined as no movement within 10 s after stimulation with glass droppers, was taken as the endpoint.

2.4. Acute toxicity test for the copepod

Standard 96-h acute toxicity tests were carried out with adult copepods. Briefly, 10 adult copepods were randomly assigned into 4 mL of test solutions ($n = 6$) including control (FASW: 0.45 μm filter membrane; 32.5 ± 1‰) and solvent control (0.01% DMSO in FASW). The temperature and light cycle during the experiment were the same as rotifer toxicity test. The exposure was hemi-static with a water renew after 48 h. No feeding was applied during the exposure. Mortality was applied as the endpoint whereby a copepod was considered dead when its urosome was at a right angle to the prosome (Finney, 1979).

2.5. Acute toxicity test for the marine medaka fish

The marine medaka fish *O. melastigma* were obtained from an established fish culture in School of Biological Sciences, the University of Hong Kong. Standard toxicity test with fish larvae (<72-h old) were applied in this experiment. Ten fish larvae were exposed to 20 mL of test solutions including control and solvent control (same as above toxicity test for copepod) in 50-mL glass beakers ($n = 3$). The exposure was conducted for 96 h in an environmental chamber with the same conditions as above. Test solutions were changed once after 48 h. No feeding was applied during the exposure.

Please cite this article as: Yi, X., et al., Binary mixture toxicities of triphenyltin with tributyltin or copper to five marine organisms: Implications on environmental risk ..., Marine Pollution Bulletin (2017), http://dx.doi.org/10.1016/j.marpolbul.2017.02.031