Marine Environmental Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Environmental Research

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

Exposure to sublethal concentrations of tributyltin reduced survival, growth, and 20-hydroxyecdysone levels in a marine mysid

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ARTICLE INFO

Keywords:
Marine mysid
Tributyltin
Growth
Survival
20-hydroxyecdysone

ABSTRACT

Tributyltin (TBT) is as an antifouling organotin compound used in boat paints. Although organotin-based antifouling agents have been banned on a global scale, the mode of action of TBT has been studied in numerous aquatic species because of its toxicity, persistence, bioaccumulation potential, and endocrine-disrupting characteristics. In this study, we conducted 96-h acute toxicity tests wherein we exposed juvenile and adult marine mysids to waterborne TBT. Over 4 weeks of exposure, mortality was dose-dependently increased in juveniles and adult mysids. To test sublethal effects of TBT on juvenile development, newborn juvenile mysids were exposed to 1, 5, or 10 ng L^{-1} TBT for 4 weeks. Subsequently, we measured morphological growth parameters and quantified the hormone ecdysterone (20-hydroxyecdysone: 20E), which controls molting in mysids. The lengths of the whole body, antennal scale, exopod, endopod, and telson were significantly smaller in the 5 and/or 10 ng L^{-1} TBT-exposed juvenile mysids than in control and DMSO-exposed groups. Levels of 20E were significantly lower at 5 and 10 ng L^{-1} TBT exposures. Additionally, the number of newly hatched juveniles was significantly lower from females previously exposed to 10 ng L^{-1} TBT. Our results indicate sublethal concentrations of TBT have inhibitory effects on the survival, growth, and production of juveniles. The lower 20E levels could be strongly associated with TBT-triggered inhibition.

1. Introduction

Tributyltin [TBT: $(C_4H_9)_3Sn^+$], is an organotin compound in anti-fouling paints that has been applied globally by the shipping industry to control fouling organisms such as barnacles and seaweed on the hulls of large ships (Fent, 1996; Hoch, 2001; Novelli et al., 2002; Sarkar et al., 2006; Antizar-Ladislao, 2008). The use of TBT as a biocide has also expanded to industrial and agricultural sectors (e.g. bactericide, fungicide, and insecticide) (Hoch, 2001; EPA, 2003; Antizar-Ladislao, 2008; Okoro et al., 2011; Shue et al., 2014). As a result of its widespread use, hydrophobicity, persistence, bioaccumulation potential, and endocrine-disrupting characteristics, TBT has become a major contaminant in marine and freshwater ecosystems (Fent, 1996; Hoch, 2001; Novelli et al., 2002; Antizar-Ladislao, 2008). Specifically, TBT is most commonly found in coastal areas such as in sediment near harbors, fishery ports, marinas, and shipyards (Harino et al., 2007; Antizar-Ladislao, 2008; Garg et al., 2011; Batista et al., 2016).

Because TBT is toxic to organisms in several environments, the International Maritime Organization (IMO, 2001) had come forward to enact a global ban of organotin compounds in anti-fouling systems under the International Convention on the Control of Harmful Anti-fouling Systems on Ships in 2008. Worldwide, more than 70 countries have agreed to ban TBT use in aquatic applications (IMO, 2001). Although TBT was banned in many countries, it is still being used as a biocide in most of the developing countries that have not joined the International Maritime Organization (Antizar-Ladislao, 2008; Costa et al., 2013). Further, TBT is still detected in water samples near harbors at concentrations up to 200–400 ng L^{-1} (Sousa et al., 2009; Radke

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https://doi.org/10.1016/j.marenvres.2018.06.006
Received 13 March 2018; Received in revised form 4 June 2018; Accepted 4 June 2018

et al., 2013) and in marine sediments up to 1–10 μg g^{-1} (Radke et al., 2013; Briant et al., 2013).

Tributyltin is a lipid soluble compound that can adsorb to fatty tissues of marine biota (Cole et al., 2015; Park et al., 2016). Once TBT enters an ecosystem, waterborne TBT can cause acute and chronic biological effects on non-target organisms, including humans. The endocrine-disrupting effects of TBT primarily occur on reproduction, immunity, and sexual development (Matthiessen and Gibbs, 1998). Additionally, TBT can cause nervous system impairment, hepatotoxicity, and behavioral changes in many organisms (Fent, 1996; Hoch, 2001; Novelli et al., 2002; Antizar-Ladislao, 2008; Yeo et al., 2017). Even at low concentrations (< 1 ng L^{-1}), TBT compounds can have many harmful effects on marine organisms, such as imposex in gastropod species, malformation of bivalves and fishes, and the inhibition of growth in algae, barnacles, oysters, and mussels (Smith, 1971; Gibbs and Bryan, 1996; Alzieu, 2000; Webster et al., 2003; Amara et al., 2018). Furthermore, humans are exposed to TBT through the consumption of contaminated organisms (e.g. fish, shrimps, oysters, mussels, and clams), drinking water, and meat. As a result, the European Water Framework Directive (WFD, 2000/60/CE) has classified TBT as a priority hazardous substance.

The ecotoxicity and mode of action of TBT has been studied in many aquatic animals (Fent, 1996; Hoch, 2001; Novelli et al., 2002; Antizar-Ladislao, 2008). Although acute or chronic toxicity values were reported in mysid species such as *Acanthomysis sculpta* (Davidson et al., 1986), *Mysidopsis bahia* (Goodman et al., 1988), and *Neomysis integer* (Verslycke et al., 2003), studies on specific effects of TBT on mysid growth remain scarce. The genus *Neomysis* contains relatively small-sized crustaceans that are commonly found in most aquatic environments, such as brackish, estuarine, coastal, and oceanic environments. For more than 20 years, the genus *Neomysis* has served as an ecotoxicology model taxonomic group because of its ease of culturing and handling in the laboratory, wide geographical distribution, short lifespan, and physiological sensitivity to various environmental factors (EPA, 2002a; Verslycke et al., 2004; Verslycke et al., 2007; Hirano et al., 2009; Min et al., 2018). *Neomysis awatschensis* (Brandt, 1851) has relatively fast growth rate with high productivity through marsupium in *Neomysis* species (Yamada et al., 2007). They are distributed widely along the estuarine and shallow coastal waters of northwestern subarctic Pacific region and northeast Asia (e.g. China, Japan, Korea). Particularly, *N. awatschensis* is increasingly being utilized as a commercially important food resource for aquaculture, fisheries, and public aquariums.

Bioassays play an important role in providing information about the impact of emerging environmental contaminants on aquatic organisms and also for monitoring water quality. Assessing acute and chronic effects of TBT in the laboratory is important for understanding the potential toxicity of TBT to mysids populations in marine environments. In crustaceans, molting is regulated by molt-promoting steroid hormones such as ecdysteroids. Ecdysterone (20-hydroxyecdysone: 20E) is an ecdysteroid hormone, secreted by the Y-organ (Goodwin, 1978; Subramoniam, 2000). Typically, 20E controls many physiological functions such as ovarian maturation, growth, molting, and reproduction. Thus, measurement of 20E levels could help determine the influence of potential endocrine-disrupting chemicals (EDC) on growth parameters in crustaceans, including mysids (Hirano et al., 2009). Consequently, the present study was aimed to examine the acute and sublethal effects of TBT on the survival, growth, and 20E levels in the marine mysid, *N. awatschensis*, at different TBT concentrations.

2. Materials and methods

2.1. Mysid culture

The *N. awatschensis* individuals used in this study were originally collected by aquarist Mr. Il-Ro Lee from the Ganghwa Island, South Korea. The species identity was verified by morphological assessment and sequence analysis of mitochondrial DNA cytochrome oxidase 1 (*CO1*) using a universal primer set (Forward primer: 5′ ggt caa caa atc ata aag ata ttg g 3'; Reverse primer: 5′ taa act tca ggg tga cca aaa aat ca 3'; Folmer et al., 1994). Approximately 5000 adults (both sexes) were provided by Mr. Lee, and we continuously cultured them in an automated aquaculture system [16 h light/8 h dark photoperiod; 20 °C; 30 practical salinity unit (psu); pH 8.0; dissolved oxygen (DO) level 7 mg L^{-1}; Incheon National University, Incheon, South Korea] using artificial seawater (TetraMarine Salt Pro, Tetra, Cincinnati, OH, USA). Temperature, salinity, pH, and DO were monitored using a portable Orion Star meter (520M-01A, Thermo Fisher Scientific Inc., MA, USA) equipped with pH/DO/conductivity electrodes. The mysids were fed daily with *Artemia salina* nauplii at a rate of 100–150 *Artemia* nauplii per mysid (SERA Artemia, Salt Lake, UT, USA). To prevent salinity fluctuation, the *Artemia* nauplii were concentrated and moved into 30 psμ artificial seawater before feeding. All the experiments involving mysids were approved by the animal care and use committees of the Incheon National University and Korea Institute of Ocean Science and Technology (KIOST).

2.2. Tributyltin exposure and toxicity test

Aqueous static renewal 96-h bioassays were performed on different stages of *N. awatschensis*. Tributyltin chloride (TBTCl) was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA; 96% purity) and was dissolved with 10^6 μg L^{-1} in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Inc., St. Louis, MO, USA) as a vehicle. Working solutions at designated nominal concentrations were prepared by diluting stock solution in artificial seawater. The concentration of DMSO in the solvent control was 0.1%.

To measure acute toxicity value, thirty younger juvenile (< 24 h after hatching), thirty older juvenile (≈7 days after hatching; DAH), or adult (≈30 DAH) mysids were treated with each concentration of TBT for 96 h. All mysid stages used in this study were originated from the 3rd generation of laboratory cultured *N. awatschensis* to ensure same developmental conditions such as age, appearance, and size. The EPA method (2016) suggested that if more than 5% of the culture or parental stock dies or shows signs of disease or stress (e.g. discoloration, unusual behavior, immobilization) during the 48 h preceding the test, mysids should not be used for a test. In our culture conditions, approximately 3–4% of mysids showed abnormalities or died. The criteria for selection of two juvenile stages were followed two EPA methods (2002b, 2016). Entire abnormal or injured mysids were discarded from toxicity test. A range-finding test as a preliminary study was conducted to establish the appropriate test solution concentrations using a wide range of TBT concentrations (i.e. 1, 10, 50, 100, 250, 500, 750, 1000, 1250, 1500, 1750, and 2000 ng L^{-1}). In a definitive test, the following concentrations of TBT were used: 0.01, 0.1, 1, 5, 10, 50, 100, 200, 400, 600, 800, 1000, 1200, and 1400 ng L^{-1}. In each stage, thirty mysids were separated into three groups for each concentration (n = 10 per each group as triplicate). Acclimation was conducted for 3 h, as the artificial seawater conditions were same with culture conditions [e.g. 16 h L/8 h D; 20 °C; 30 psμ; pH 8.0; (DO) level > 7 mg L^{-1}]. Temperature, salinity, pH, and DO of stock artificial seawater were daily monitored using a portable Orion Star meter (Thermo Fisher Scientific Inc.).

Ten mysids were distributed to 500 ml test vessel (Duran, Germany) containing 300 ml of solvent control (DMSO) or toxicant solution. All test vessels and retention chambers were identical. Half of the test solutions were refreshed every 24 h with an addition of equivalent concentration of DMSO or TBT. Mysids were periodically observed during the toxicity test, and dead mysids and debris were quickly removed. Basically dead mysids lack of visible movement or response to gentle prodding, as described in the EPA method (2016). They were fed daily with less than 24 h old *A. salina* nauplii at a rate of 100 *Artemia* nauplii per mysid. The *Artemia* nauplii were concentrated into 50 ml and the