Ecotoxicology and Environmental Safety 147 (2018) 275–282

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Multigenerational effects of triclosan on the demography of *Plationus patulus* and *Brachionus havanaensis* (ROTIFERA)

Brenda Karen González-Pérez[a], S.S.S. Sarma[b,*], M.E. Castellanos-Páez[a], S. Nandini[b]

[a] Programa de Doctorado en Ciencias Biológicas y de la Salud. Universidad Autónoma Metropolitana, Unidad Xochimilco, Calzada del Hueso, No. 1100, Villa Quietud, Mexico City, Código Postal-04960, Mexico

[b] Universidad Nacional Autónoma de México, Campus Iztacala, Av. de los Barrios #1, Col. Los Reyes, Iztacala, Tlalnepantla, State of Mexico C.P. 54090, Mexico

ARTICLE INFO

Keywords:
Endocrine disruptor
Ecotoxicology
Life table demography
Zooplankton

ABSTRACT

Triclosan is a personal care product widely used in North America, Europe and Asia as antimicrobial ingredient in many consumer chemical products. In Mexico concentrations of triclosan have been reported in aquatic systems. However, there is no law regulating the presence of chemicals such as triclosan, in aquatic systems. The scarce data about this chemical has increased concern among ecotoxicologists regarding possible effects on aquatic organisms. Moreover, multigenerational studies are rarely studied and the results vary depending on the contaminant. Rotifers, are a dominant group of zooplankton, and have been used in aquatic risk assessments of personal care products due to their sensitivity and high reproductive rates. *Plationus patulus* and *Brachionus havanaensis* are common rotifers distributed in aquatic ecosystems of Mexico and have been used in ecotoxicological bioassays. In this study, the median lethal concentration (LC50, 24 h) of *P. patulus* and *B. havanaensis* exposed to triclosan was determined. Based on the LC50, we tested three sublethal concentrations of triclosan to quantify the demographic responses of both rotifers for two successive generations (F0, and F1). The 24 h LC50 of triclosan for *P. patulus* and *B. havanaensis* were 300 and 500 μg L^{-1} respectively. Despite the concentration, triclosan had an adverse effect on both *Plationus patulus* and *Brachionus havanaensis* in both generations exposed. Experiments show that *P. patulus* was more sensitive than *B. havanaensis* when exposed to triclosan. When exposed to triclosan the parental generation (F0) of *P. patulus* was far more affected than F1.

1. Introduction

Concentrations of emerging contaminants in freshwater ecosystems is on the rise (Murray et al., 2010). Thousands of chemicals which are widely used in human and animal health care products have been classified as endocrine disruptors (Frey et al., 2011). Endocrine disruptors are natural or synthetic compounds that can alter the hormonal and homeostatic systems which facilitate organisms to communicate and respond to the environment (USEPA, 2002; Diamanti-Kandarakis et al., 2009).

Endocrine disruptors from different sources, in many cases, are discharged directly into reservoirs, without prior treatment. Some of these are found in concentrations that range from ng L^{-1} to μg L^{-1} in effluent waters and may have negative effects on non-target organisms (Daughton and Ternes, 1999; Jjemba, 2006). In spite of their low concentrations in the medium, their continuous release into aquatic environments can result in undesirable effects on aquatic organisms (Cunningham et al., 2009). Wastewater treatment plants (WWTP) are not fully equipped to eliminate most of the endocrine disruptors. Hence, the quality of water that has been released from WWTP has become questionable in many countries including Mexico (Liu et al., 2009; Nandini et al., 2016).

The US Environmental Protection Agency (EPA) has developed a strategy for a continuous monitoring of substances with potential for endocrine disruption (EDSTAC, 1996). There are also guidelines which contain data on the permissible levels of different substances including endocrine disruptors to protect aquatic systems (ISTAS, 2002). However, rigorous laws do not exist in Mexico, especially regarding the maximum permissible limits of different substances such as endocrine disruptors, in aquatic systems. In spite of the fact that several water bodies in Mexico receive partially treated waste waters continually, little information is available on the concentrations of endocrine disruptors in aquatic ecosystems in the country (Gibson et al., 2007, 2010).

Triclosan (2, 4, 4′-thrichloro-2′-hydroxy- diphenyl ether) is a personal care product widely used in North America, Europe and Asia as antimicrobial ingredient in antibacterial soaps (bars and liquids), deodorants, body creams, cosmetics, antiseptic products and toothpaste

* Corresponding author.
E-mail address: sarma@unam.mx (S.S.S. Sarma).

http://dx.doi.org/10.1016/j.ecoenv.2017.08.049
Received 25 July 2017; Received in revised form 12 August 2017; Accepted 21 August 2017

(McAvoy et al., 2002; Sabaliunas et al., 2003; EU, 2007). Endocrine disrupting effects have been associated to triclosan due to its potential ability to mimic as estrogens, androgens or even thyroid hormones (Dann and Hontela, 2011). Triclosan can be transformed into other products under certain conditions of temperature, pH and sunlight, which can bioaccumulate (Orvos et al., 2002; Latch et al., 2003).

Foran et al. (2000) observed changes in the sex ratio of Japanese Medaka fish (*Oryzias latipes*) when exposed to triclosan. There a few studies which report that triclosan has an adverse effect on microalgal species too (Orvos et al., 2002; Delorenzo et al., 2008). The data are still scarce on the effects of endocrine disruptors including triclosan, to other species of plankton (Burkhardt-Holm, 2010). Freshwater ecosystems including rivers, ponds, lakes and man-made systems such as wastewater treatment plants contain several organisms including zooplankton (Wallace and Snell, 2010; Basumatary et al., 2017). Rotifers, which are a numerically dominant group of zooplankton, have been used in risk assessments of personal care products (PCPs) due to their great sensitivity and high reproductive rates (Snell and Joaquim-Justo, 2007). They were also found to be more sensitive to some disinfectants than other invertebrates (Zhang et al., 2016). Xenobiotics adversely affect not only in the exposed organisms but also their offspring (Brennan et al., 2006). Hence, it is important to evaluate two or more life cycles of the test organisms to determine the multigenerational effects of these chemicals (Marcial and Hagiwara, 2007).

Multigenerational studies are rarely studied because of time constraint (Galusa et al., 2014) but their outcomes vary widely depending on the generation (Biron et al., 2012). The effects of potential transfer of pollutants from mothers to their offspring or maternal effects are ignored due to the fact that ecotoxicological studies mainly focus on the effects of neonates from unexposed mothers (Guo et al., 2012).

Members of the genus *Brachionus*, mainly *B. plicatilis*, and *B. calyciflorus* have already been used as bioassay organisms for testing several xenobiotics (Snell and Janssen, 1995). Nonetheless, *Brachionus havanaensis* is a common rotifer in freshwater ecosystems of Mexico and has also been used in ecotoxicological bioassays (Nandini et al., 2005). *Plationus patulus* is widely distributed and has been recognized as a standard test species by the American Public Health Association (Sarma et al., 2014; Martínez-Gómez et al., 2015).

The aim of this study is therefore to test the acute and chronic effects of triclosan on the life table demography of two rotifers species *P. patulus* and *B. havanaensis* during two successive generations.

2. Methods

The rotifers *Plationus patulus* and *Brachionus havanaensis* were isolated from Lake Xochimilco, a UNESCO heritage waterbody in Mexico City (Garza-Mouriño and Castellanos-Paéz, 2003). Monoclonal populations were established using the single-celled green alga, *Chlorella vulgaris* as food and on synthetic medium, moderately hardwater (EPA medium). The EPA medium was daily prepared by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl in 1 L of distilled water (Weber, 1993). *Chlorella vulgaris* was batch-cultured using Bold's basal standard medium (Borowitzka and Borowitzka, 1988). Log phase algae were harvested and concentrated by centrifugation at 3000 rpm for 5 min and repeatedly rinsed in distilled water. The concentrated algae were resuspended in 5 mL of distilled water. The algal density was estimated using haemocytometer. Cultures and experiments were maintained at a temperature of 22 ± 2 °C, pH 7.0–7.4, and fluorescent illumination was continuous but diffuse.

To obtain neonates of known age, we separated a large number of egg-bearing individuals of each species from the mass culture jars in exponential phase of growth. Neonates hatched within 2–6 h were used in the experiments. Analytical grade Triclosan was purchased from Sigma Aldrich (USA, Lot: LRAA1072) and was dissolved in distilled water for a stock solution of 2 mg L^{-1}. The concentrations were adjusted after confirmation from HPLC.

2.1. Acute toxicity tests

Range finding tests for both *P. patulus* and *B. havanaensis* were conducted exposing populations to triclosan based on the values from the literature; from 0 to 400 μg L^{-1} for *P. patulus* and 0–500 μg L^{-1} for *B. havanaensis*. Acute toxicity tests were conducted at five concentrations: 0, 50, 100, 200, 300 and 400 μg L^{-1} for *P. patulus* and; 0, 100, 200, 300, 400 and 500 μg L^{-1} for *B. havanaensis*. Standard acute toxicity methods for *Brachionus* from the American Society for Testing and Materials (ASTM) were used (ASTM, 1998). The number of deaths were recorded after 24 h. Median lethal concentration (LC_{50}) was calculated using the Probit method (Finney, 1971).

2.2. Chronic toxicity tests

Standard cohort life table experiments were conducted at 3 sublethal concentrations of triclosan and control group (without the toxicant) for both *P. patulus* and *B. havanaensis* separately (Krebs, 1985). *Chlorella vulgaris* was used to feed the rotifers in the test jars at a density of 1×10^6 cells mL^{-1} per day. Toxicity tests were performed in two parts using two generations (F0 and F1). The first experiment was initiated with F0 for which the experimental design consisted of 20 transparent jars of 50 mL capacity, each with 20 mL of test medium. Each treatment had 5 replicates. Pasteur pipettes were used to individually count and introduce 20 neonates of each rotifer species into the test jars containing 20 mL EPA medium with *Chlorella vulgaris* and one of the chosen concentrations of triclosan. The second experiment consisted in life table demography of the next generation (F1) of neonates from each rotifer species, taken from the first batch of offspring at the lowest concentration tested. At the higher test concentrations, the number of offspring produced was low and insufficient to set up an experiment with the F1 generation.

From each test jar the number of neonates produced and dead adults, when present, were counted and discarded after every 12 h. The surviving individuals from each cohort were daily transferred to fresh jars containing triclosan and food concentration. Experiments for both *P. patulus* and *B. havanaensis* were discontinued when every individual from each cohort had died. To derive the life table variables, standard formulae was used (survivorship, fecundity, average lifespan and gross, net reproductive rates, generation time and rate of population increase) (Krebs, 1985).

- l_x = Proportion of surviving at the start of age x
- m_x = Offspring produced per female at age x

Life expectancy at birth x: $e_x = \frac{T_x}{n_x}$

where, T_x = cumulative number of individuals from the age x

- n_x = number living at the age x (days)

Net reproductive rate $R_o = \sum_0^\infty l_x \cdot m_x$

Generation time : $T = \frac{\sum l_x \cdot m_x \cdot x}{R_o}$

Rate of population increase per day, $r\,1 = \Sigma e^{-rx} l_x m_x$
(Euler-Lotka equation, solved iteratively).

Results were analyzed using two-way analysis of variance (ANOVA) and post-hoc (Tukey tests) to determine whether there were significant differences between each treatment (Sokal and Rohlf, 2000).

3. Results

The age specific survivorship (lx) curves for *Plationus patulus* and *Brachionus havanaensis* exposed to triclosan declined with increasing