Marine Pollution Bulletin xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Triclosan alterations of estuarine phytoplankton community structure

James L. Pinckney *, Laura Thompson, Sarah Hylton

Marine Science Program and Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

ARTICLE INFO

Article history:
Received 4 November 2016
Received in revised form 20 March 2017
Accepted 25 March 2017
Available online xxxx

Keywords:
ChemTax
Photopigments
Pharmaceutical
Antimicrobial
Estuary
South Carolina

ABSTRACT

Antimicrobial additives in pharmaceutical and personal care products are a major environmental concern due to their potential ecological impacts on aquatic ecosystems. Triclosan (TCS) has been used as an antiseptic, disinfectant, and preservative in various media. The sublethal and lethal effects of TCS on estuarine phytoplankton community composition were investigated using bioassays of natural phytoplankton communities to measure phytoplankton responses to different concentrations of TCS ranging from 1 to 200 μg l^{-1}. The EC_{50} (the concentration of an inhibitor where the growth is reduced by half) for phytoplankton groups (diatoms, chlorophytes, cryptophytes) examined in this ranged from 10.7 to 113.8 μg TCS l^{-1}. Exposures resulted in major shifts in phytoplankton community composition at concentrations as low as 1.0 μg TCS l^{-1}. This study demonstrates estuarine ecosystem sensitivity to TCS exposure and highlights potential alterations in phytoplankton community composition at what are typically environmental concentrations of TCS in urbanized estuaries.

1. Introduction

Antimicrobial additives in pharmaceutical and personal care products are a major environmental concern due to their potential ecological impacts on aquatic ecosystems (Orvos et al., 2002; Dann and Hontela, 2011; Brausch and Rand, 2011; Bedoux et al., 2012). Triclosan (TCS) is a commonly used antimicrobial agent in soaps, toothpaste, and a variety of personal care products (Dann and Hontela, 2011). The primary mode of action for TCS is as a fatty acid biosynthesis inhibitor. With an estimated daily use of 1500 kg of TCS per day (>300 tons y^{-1}) in the US, this biocide is often found in treated wastewater effluents and sludge as well as surface waters and sediments (Wilson et al., 2008; Kumar et al., 2010; Bedoux et al., 2012). Sewage effluent may have TCS concentrations as high as 5.4 μg l^{-1} (Kumar et al., 2010). In a recent study of freshwater rivers in the US, the US Geological Survey (USGS) identified TCS as one of the most frequently detected xenobiotic compounds, with surface water concentrations up to 2.3 μg l^{-1} (Kolpin et al., 2002). Ecological risk assessments (ERA) for such compounds are crucial for demonstrating lethal and sublethal effects and assessing the potential negative impacts on phytoplankton in estuarine habitats (Reiss et al., 2009).

Triclosan is also known by the trade names of Irgasan DP300, Biofresh, and Microban (Adolfsson-Erici et al., 2002). Since 1968, TCS has been used as an antiseptic, disinfectant, and preservative in various media including cosmetics, household cleaning products, and toys, and imbedded in plastics used for medical devices, textiles, and kitchen utensils (Dann and Hontela, 2011; Bedoux et al., 2012). TCS has a low water solubility (12 mg l^{-1}, Reiss et al., 2002) and a half-life of 8 and 4 days in freshwater and seawater, respectively (Aranami and Readman, 2007). Because of its hydrophobicity, TCS readily adsorbs to particles and surfaces and accumulates in sediments (Lindström et al., 2002; Wilson et al., 2008). In the natural environment, TCS can be slowly photodegraded and biodegraded to form methyl-triclosan (Reiss et al., 2002). Methyl-triclosan resists photodegradation and has a higher potential to bioaccumulate since this form of TCS is lipophilic (Lindström et al., 2002; Orvos et al., 2002; Dann and Hontela, 2011). Triclosan is regulated by both the US Environmental Protection Agency (USEPA) (for pesticide uses) and the US Food and Drug Administration (USFDA) (for non-pesticide uses). In September 2016, the USFDA banned the use of TCS from over-the-counter antibacterial hand and body washes (21 CFR Part 310, 06/09/2016).

The primary pathway for TCS to enter surface waters is via wastewater treatment plant effluent at concentrations that can exceed 40 μg l^{-1} (Coogan et al., 2007; Kumar et al., 2010). Environmental concentrations of TCS in surface waters range from trace amounts to 10 μg l^{-1} (Wilson et al., 2008; Brausch and Rand, 2011; Bedoux et al., 2012). In Charleston Harbor, SC, limited sampling reported a maximum concentration of TCS of 0.014 μg l^{-1} (DeLorenzo et al., 2008; Fair et al., 2009). Similar concentrations (0.005 μg l^{-1}) have been measured in the nearby Savannah, Ogeechee, and Vernon Rivers (Georgia) (Kumar et al., 2010). TCS can be persistent in the environment, with a half-life of >11 days (Bester, 2005). Although these concentrations seem quite low, sublethal effects of TCS have not been examined for marine microbiota in natural systems.

* Corresponding author at: Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA.
E-mail address: pinckney@sc.edu (J.L. Pinckney).

http://dx.doi.org/10.1016/j.marpolbul.2017.03.056
0025-326X/

The environmental impacts of TCS have not been well-studied in estuarine systems (DeLorenzo et al., 2008). However, there is clear evidence that this compound is extremely toxic to aquatic microbiota at μg l^{-1} concentrations (Orvos et al., 2002, DeLorenzo et al., 2008, Brausch and Rand, 2011). Unialgal cultures of microalgae, including phytoplankton, are highly sensitive to TCS concentrations as low as 0.5 μg l^{-1} (Orvos et al., 2002, DeLorenzo et al., 2008). This high sensitivity is likely due to disruption of lipid synthesis pathways (McMurry et al., 1998; Lu and Archer, 2005), membrane destabilization (Lyrge et al., 2003; Franz et al., 2008), or uncoupling of oxidative phosphorylation (Newton et al., 2005). Studies on unialgal cultures suggest that TCS may bioaccumulate within plant cells (Coogan et al., 2007). Another consequence is that the presence of these biocides may have a selective effect on phytoplankton composition, depending on sensitivity. The implications for providing a foothold for exotic or invasive species of phytoplankton or promoting the growth and proliferation of harmful algal species are important to consider and explore.

The primary purpose of this research was to investigate the sublethal and lethal effects of TCS on relatively pristine estuarine phytoplankton communities at environmentally-relevant concentrations. We determined the effective concentrations for 50% mortality (EC_{50}) for different phytoplankton taxa and concentration effects on phytoplankton community composition for two different estuaries. The quantitative investigation of the effects of TCS in estuarine ecosystems is crucial for ecological risk management and for providing evidence needed to support the establishment and enactment of regulatory guidelines and laws.

2. Materials and methods

North Inlet Estuary, near Georgetown, South Carolina, USA is a euhaline *Spartina* marsh system with minimal anthropogenic impacts (Allen et al., 2014) (http://www.northinlet.sc.edu). The nearly pristine conditions of this estuary minimize potential experimental artifacts due to acclimation of the local phytoplankton communities to chronic antibiotic exposure (Wirth et al., 1998; Sanger et al., 1999). Winyah Bay, also near Georgetown, SC, is a river-dominated estuary that receives input from the Pee Dee River which drains a mostly rural (agricultural and forest lands) watershed of 4.7 million ha. A complete ecological and environmental description of the North Inlet – Winyah Bay System can be found in Allen et al. (2014).

Bioassays were used to measure phytoplankton responses to different concentrations of TCS. Seawater containing natural phytoplankton communities was collected in October 2015 and February 2016 at high tide from Oyster Landing in North Inlet Estuary (33.3341° N, 79.1929° W) and at the Georgetown Marina in Winyah Bay (33.3652° N, 79.2663° W). Salinities at the time of water collection for these experiments were ca. 6 and 31 ppt for Georgetown Marina and Oyster Landing, respectively. Collected water was dispensed into 250 ml clear polystyrene culture flasks (VWR, cat. # 10062–862).

Triclosan (5-Chloro-2-(2,4-dichloro-phenoxy) phenol; $C_{12}H_7Cl_3O_2$; Alfa Aesar cat. # L18655) was dissolved in hplc-grade acetone to make stock solutions (30–3000 μg TCS ml^{-1}) then added to the experimental treatments to achieve final concentrations of 1, 2.5, 5, 10, 15, 25, 50, 75, 100, and 200 μg TCS l^{-1}. There were 5 replicates for each concentration. Controls consisted of sample water without any addition of TCS. Preliminary experiments indicated that the addition of up to 20 μl of acetone (the carrier for this experiment) had no effect on phytoplankton responses. Nitrate and phosphate (20 μM $NaNO_3$, 10 μM KH_2PO_4 final concentrations) were added to all treatments including the control to minimize nutrient limitation during the bioassays.

Light for incubations was supplied using a 91 cm, 4 × 39 W Ocean Light T5 hood (10,000 K 39 W –TRU fluorescent lamps) to achieve an irradiance of ca.130 μmol quanta m^2 s^{-1}. Light was cycled according to times of sunrise and sunset on the dates the water samples were collected. Temperature was a constant 22 °C. Bioassays were incubated for a period of 72 h. At the end of the incubations, samples were vacuum (−50 kPa) filtered onto Whatman GF/F glass microfiber filters. One half of the sample was pre-filtered through 20 μm nitex mesh to size fractionate samples. Thus each sample was divided into two size fractions; phytoplankton <20 μm in size and whole water.

Phytoplankton photopigment concentrations were measured using HPLC (Roy et al., 2011). Filters were first lyophilized for 18–24 h at −50 °C. Photopigments were then extracted by adding 750 μl of 90% aqueous acetone solvent followed by storage for 12–20 h at −20 °C. Filtered extracts (250 μl) were injected into a Shimadzu HPLC with a single monomeric column (Rainin Microsorb, 0.46 × 1.5 cm, 3 μm packing) and a polymeric (Vydac 201TP54, 0.46 × 25 cm, 5 μm packing) reverse-phase C18 column in series as the solid phase. A non-linear binary gradient of solvent A (80% methanol: 20% 0.5 M ammonium acetate) and solvent B (80% methanol: 20% acetone) was used for the mobile phase (Pinckney et al., 2001). Absorption spectra and chromatograms (440 ± 4 nm) were obtained using a Shimadzu SPD-M10av photodiode array detector and pigment peaks were identified by comparing retention times and absorption spectra with pure standards (DHI, Denmark). The synthetic carotenoid β-apo-8′-carotenal (Sigma) was used as an internal standard.

The software ChemTax (v. 1.95; Mackey et al., 1996, Wright et al., 1996) was used to determine the relative abundance of major phytoplankton groups (Pinckney et al., 2001; Lewitus et al., 2005; Higgins et al., 2011). The initial pigment ratio matrix used for this analysis was a combination of matrices provided by Mackey et al. (1996), Lewitus et al. (2005), and Schlüter et al. (2000). The convergence procedure outlined by Latasa (2007) was used to minimize errors in algal group biomass due to inaccurate pigment ratio seed values. A two-step cluster analysis procedure based on log-likelihood distance measures of ten photopigment variables was used to define homogeneous groups for separate bins in ChemTax analyses (SPSS v. 24). Four clusters, each consisting of 220 (49%), 103 (23%), 73 (16%), and 57 (13%) samples were constructed in the analysis. The groups were analyzed using the four analysis bins in ChemTax to provide estimates of the relative abundances of 5 algal groups (chlorophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates) in units of μg chlorophyll *a* l^{-1}.

The percent inhibition (%inhibition) for algal groups in each sample was calculated using the equation:

$$\%\text{inhibition} = 100\left(1 - \frac{r_{\text{treatment}}}{r_{\text{control}}}\right)$$

Where $r_{treatment}$ and $r_{control}$ are the algal group abundances in the treatment and the corresponding control, respectively. Algal responses to the TCS additions were fit to a hyperbolic Hill equation in the form of:

$$y = I_{max}\left(\frac{x^n}{(K_I^n + x^n)}\right)$$

Where *y* is the %inhibition, x is the concentration (μg l^{-1}) of TCS, I_{max} is the maximum %inhibition, K_I is the TCS concentration at which the percent inhibition is one-half of the maximum %inhibition, and *n* is the Hill coefficient. Non-linear curve-fitting was accomplished using an iterative Levenberg-Marquardt procedure (OriginPro 2016).

3. Results

Photopigment concentrations were analyzed in 4 separate bins in ChemTax with the resulting RMS errors of 0.082, 0.082, 0.050, and 0.041. The three most abundant groups for the Oyster Landing samples were diatoms, chlorophytes, and cryptophytes which composed ca. 80% of the phytoplankton community. For the Marina location, diatoms and chlorophytes were the primary constituents of the community (ca. 90%). Low concentrations of dinoflagellates and cyanobacteria were detected, but, due to their low abundances, were excluded from

Please cite this article as: Pinckney, J.L., et al., Triclosan alterations of estuarine phytoplankton community structure, Marine Pollution Bulletin (2017), http://dx.doi.org/10.1016/j.marpolbul.2017.03.056