Research article

Effect of triclosan and its photolysis products on marine bacterium *V. fischeri* and freshwater alga *R. subcapitata*

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A R T I C L E   I N F O

A B S T R A C T

The use of antibacterial agents in consumer products may lead to adverse effects in waters receiving treated wastewater. Triclosan is one of the antibacterial agents used widely in the world and its high usage leads to relatively high concentrations in wastewater effluents. In this study, the probable effect of triclosan in receiving waters was assessed using different organisms. The EC50 values were $668 \pm 80\,\mu g/L$ and $7.8 \pm 0.1\,\mu g/L$, for *Vibrio fischeri* and *Raphidocelis subcapitata*, respectively, indicating the higher sensitivity of the alga. The toxicity of triclosan upon exposure to UV light decreased for both species, as suggested by the increase in EC50 values ($1300 \pm 50\,\mu g/L$ and $8.7 \pm 0.6\,\mu g/L$ for *V. fischeri* and *R. subcapitata*, respectively). The effect of photolysis on toxicity reduction was higher for *V. fischeri* and the EC50 values were similar for direct and indirect photolysis. LC-MS/MS analysis of samples with and without UV exposure suggested a decrease in triclosan concentration as well as formation of photolysis byproducts upon photolysis.

1. Introduction

Triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol] is a non-ionic, broad spectrum antimicrobial agent that is used in personal care products (PCP) such as soaps, toothpaste, mouthwash, and cosmetics as well as in textiles nearly for 40 years. In the period from 1992 to 1999, the majority of the 700 antibacterial products on the market contained triclosan as an active ingredient (Schweizer, 2001) and triclosan consumption is supposed to increase 2 fold from 2008 to 2020 (Huang et al., 2014). During household use, nearly 96% of triclosan is being discharged into the sewer system. Triclosan concentration was reported to range between 0.01 and 600 μg/L in wastewater treatment plant influents (McAvoy et al., 2002; Aguera et al., 2003; Bester, 2003; Mezcua et al., 2004; Nakada et al., 2007; Kasprzyk-Hordern et al., 2008; Chalew and Halden, 2009; Bedoux et al., 2012), 0.01–22 μg/L in wastewater treatment plant effluents (McAvoy et al., 2002; Aguera et al., 2003; Heidler and Halden, 2007; Wick et al., 2010), and between 0.0003 and 2.300 μg/L in surface waters (Lindström et al., 2002; Singer et al., 2002; Morrall et al., 2004; Bester, 2005;

Halden and Paull, 2005; Kasprzyk-Hordern et al., 2008; Fair et al., 2009; Brausch and Rand, 2011; Wang et al., 2014; Hopkins and Blaney, 2016). In addition, triclosan is one of the micropollutants of which the concentration has been increasing in receiving waters (Maruya et al., 2015). When the concentration levels of triclosan in wastewater treatment plant effluents and surface water and its increasing trend were considered, adverse effects of triclosan may be observed in streams where the discharged wastewater accounts for the majority of the flow (wastewater-dominated streams).

Due to its antimicrobial properties, triclosan is expected to have a toxic effect on aquatic organisms and an inhibitory effect on activated sludge biomass (Orvos et al., 2002; Stasinakis et al., 2007, 2008; Coogan and Point, 2008; Farré et al., 2008; Fang et al., 2010). The EC$_{50}$ value for growth inhibition during 96-h exposure time to *Raphidocelis subcapitata* was reported as 4.46 μg/L (Orvos et al., 2002). Yang et al. (2008) reported the growth inhibition EC$_{50}$ value of triclosan as 0.53 μg/L for 72-h exposure time to *R. subcapitata*. Moreover, its effect can be observed at much lower concentrations (*i.e.*, 15 ng/L) for algal biofilms subjected to triclosan (Wilson et al., 2003). In addition, the accumulation of triclosan in algae (*Cladophora* spp) may lead to ecotoxicological problems (Coogan and Point, 2008).

The effect of triclosan to *Vibrio fischeri*, a marine bacteria, is three orders of magnitude lower compared with its effect on algae (*Raphidocelis subcapitata* and *Anabaena flos-aquae*) with an EC$_{50}$

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range between 280 and 1500 µg/L for 15-min exposure time (Farré et al., 2008; Stasinakis et al., 2008). Triclosan also shows inhibition on activated sludge microorganisms at higher concentrations (EC$_{50}$ values for its effect on specific oxygen uptake rate (SOUR): approximately 10000 µg/L for 15 days solids retention time (SRT)) (Stasinakis et al., 2007).

In addition to having inhibitory/toxic effects on organisms, triclosan could be degraded or converted into dioxins, especially 2,8-dichlorodibenzo-*p*-dioxin (2,8 DCDD) in wastewater and surface water samples when exposed to natural sunlight or UV light (Latch et al., 2003; Tohidi and Cai, 2015). 2,8 DCDD or other transformation byproducts may also have toxic effects on aquatic organisms, especially when the relatively short half-lives reported for triclosan photolysis are considered (Bedoux et al., 2012). Although there are several studies on the photolysis rate (Duran-Alvarez et al., 2015) or photolysis pathways of triclosan (Sanchez-Prado et al., 2006; Kliegman et al., 2013; Martínez-Zapata et al., 2013), information on the ecotoxicological effects of triclosan's photolysis products is very limited. Moreover, most of the ecotoxicological studies lack analytical measurements to address the cause of toxicity.

The aim of this study is to determine the possible adverse effects of triclosan as well as the effects of its direct and indirect photolysis products on aquatic organisms. To investigate the toxic effects of triclosan to aquatic organisms, different ecotoxicological tools consisting of species from marine and freshwater were used. Moreover, the results were coupled with liquid chromatography-tandem mass spectroscopic (LC-MS/MS) analyses to provide insights into the photolysis products of triclosan and their effects on aquatic species.

2. Materials and methods

All solvents and chemicals were of analytical grade and were purchased either from Merck or Sigma-Aldrich. Analytical grade triclosan (Sigma-Aldrich) was used to confirm presence of triclosan and optimize mass spectrometer parameters. Glassware used for toxicity experiments were washed with 1 N HCl and ultra-pure water and then sterilized in autoclave at 121 °C for 15 min before each experiment set.

2.1. Photolysis experiments

For all of the photolysis experiments, a chamber containing 254 nm UV light-emitting mercury lamps was used. There were 10 mercury lamps placed on the sides of the UV chamber providing approximately 1.5 W m^{-2}. The samples were exposed to UV light in 250 mL quartz reactors at room temperature without any pH adjustment.

During direct photolysis experiments, triclosan dissolved in ultra-pure water (0.055 µS/cm) was exposed to UV light for 3 h. The concentration ranges of triclosan used for the photolysis experiments was between 0 and 3600 µg/L and 0—20 µg/L for *Vibrio fischeri* and *Raphidocelis subcapitata* respectively. The presence of natural organic matter in surface waters may affect triclosan photolysis (*i.e.*, indirect photolysis); therefore, 12 mg/L humic acid (5 mg/L TOC equivalent to represent surface waters) was dissolved in ultra-pure water along with triclosan and this solution was exposed to UV light for 3 h for indirect photolysis experiments. Each experiment set contained a dark control (the same experimental conditions except light exposure). Samples were collected before and after UV exposure for ecotoxicological analysis and identification of photolysis byproducts.

2.2. Determination of photolysis byproducts

Thermo Electron Cooperation TSQ Quantum Access triple quadruple mass spectrometer (MS) coupled with Accela Ultra Performance Liquid Chromatograph (UPLC) with electrospray ionization interface was used for analysis of triclosan and its photolysis products. Samples were introduced directly to MS via syringe pump on the MS and analyzed in full scan mode in a range of 30—600 m/z. Triclosan and photolysis products were analyzed in negative ionization mode. Parameters used during MS analysis were as follows: Spray voltage = 3500 V, sheath gas pressure = 25 arb, and capillary temperature = 350 °C.

2.3. Vibrio fischeri toxicity experiment

V. fischeri was exposed to triclosan at different concentrations (Range of concentrations: 0—1800 µg/L with 8 samples) according to ISO 11348-2 standard using BioTox freeze-dried *V. fischeri* bioassay kit (ISO, 1998) for 15 min. Luminescence intensities were measured using AboaTox limunometer before and after exposure. We calculated the EC values via normalizing intensity difference respect to blank and fitting a non-linear regression curve to data. *V. fischeri* toxicity experiments were carried out in duplicates. The effect of humic substances (HS) and UV-photolyzed HS on the toxicity of triclosan on *V. fisheri* was checked by using blank controls and comparing the reduction of light emission.

2.4. Algal growth inhibition experiments with freshwater algae, Raphidocelis subcapitata

Determination of toxicity of triclosan to the freshwater species was examined by algal growth inhibition experiments using the species *Raphidocelis subcapitata* (obtained from the Culture Library of University of Gottingen) based on EN ISO 8692:2004 (ISO, 2004). Algal toxicity tests were carried out in 250 mL conical flasks containing 100 mL of algal growth media for 72 h. Cultures were maintained in a cabinet under continuous illumination (white fluorescent light) at 22 ± 4 °C for incubation and were mixed twice a day by hand. The algae were exposed to different concentrations of triclosan (0—10 µg/L with 7 samples) in different test flask along with a control flask containing only algal growth media at the same time period. Algal growth yield was measured by a haemocytometer as cell counts before and after exposure.

Difference between growth rate in control flask and the test flask provided inhibition rate in each flask. A non-linear regression curve using SigmaPlot 12.0 was fitted to inhibition—concentration data to calculate the EC values. Algal toxicity experiments were carried out in triplicates.

3. Results and discussion

3.1. Photolysis products of triclosan

When a solution containing only triclosan at a concentration of 4000 µg/L was exposed to 3 h UV treatment, the signal intensity of m/z: 287 or 289 indicating triclosan [M-H$^+$], decreases approximately by 40% suggesting that UV degrades triclosan. The decrease in triclosan concentration was clear from the decrease in the signal intensities (signal intensities of 500 000 vs. 280 000 in Fig. 1a and Fig. 1b, respectively) within the same sequence. Our assumption for the concentration reduction based on signal intensity is also supported by the research results of Martínez-Zapata et al. (2013) who observed a similar decrease (approximately 30%) in triclosan concentration upon 3 h photolysis with a xenon lamp at 250 W m^{-2}. In the presence of HS, the decrease in triclosan intensity was higher,