Chemosphere 93 (2013) 1009–1014

Contents lists available at SciVerse ScienceDirect

Chemosphere

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

Evaluation of food chain transfer of the antibiotic oxytetracycline and human risk assessment

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HIGHLIGHTS

- Shows for the first time that OTC is able to transfer from water to plant to fish.
- Characterizes bioconcentration of OTC by a floating aquatic plant (*Wolffia globosa*).
- Compares accumulation of OTC from water and watermeal by fish (*Probarbus jullieni*).
- Quantitative risk assessment for human consumption of contaminated fish undertaken.

ARTICLE INFO

Article history:
Received 6 January 2013
Received in revised form 16 May 2013
Accepted 25 May 2013
Available online 20 June 2013

Keywords:
Food chain transfer
Bioconcentration
Biomagnification
Oxytetracycline
Watermeal (*Wolffia globosa* Hartog & Plas)
Seven-striped carp (*Probarbus jullieni*)

ABSTRACT

There has been recent concern regarding the possibility of antibiotics entering the aquatic food chain and impacting human consumers. This work reports experimental results of the bioconcentration of the antibiotic oxytetracycline (OTC) by the Asian watermeal plant (*Wolffia globosa* Hartog & Plas) and bioaccumulation of OTC in watermeal and water by the seven-striped carp (*Probarbus jullieni*). They show, for the first time, the extent to which OTC is able to transfer from water to plant to fish and enter the food chain. The mean bioconcentration factor (dry weight basis) with watermeal was 1.28×10^3 L kg^{-1}. Separate experiments were undertaken to characterize accumulation of OTC by carp from water and watermeal. These showed the latter pathway to be dominant under the conditions employed. The bioconcentration and biomagnification factors for these processes were 1.75 L kg^{-1} and 2×10^{-4} kg g^{-1} respectively. Using an aqueous concentration range of 0.34–3.0 μg L^{-1}, hazard quotients for human consumption of contaminated fish of 1.3×10^{-2} to 1.15×10^{-1} were derived.

1. Introduction

Antibiotics are biologically active compounds categorized as emerging environmental contaminants of concern (de la Torre et al., 2012). Widespread use and poor absorption following ingestion has resulted in a ubiquitous environmental presence of antibiotics. Despite this, little is known of their fate and behavior in the aquatic environment (Xu and Li, 2010). Concern has been expressed because of the potential for antibiotics to enter and move through food chains (Boxall and Ericson, 2012). Farré and Barceló (2012) have recently noted that farmed fish and shrimp are often produced in crowded facilities with little regulation of antibiotic use and that the discovery of chloramphenicol residues in shrimp has increased worldwide public awareness.

In food chain transfer, a number of processes can occur that require explanation and delineation. In aquatic systems, plants and other organisms can bioconcentrate chemicals such as antibiotics from water. Bioconcentration is hence defined as the accumulation of a chemical from the ambient environment of an organism via its respiratory and dermal surfaces. This process is quantified by the bioconcentration factor (*BCF*), the ratio of chemical concentration in an organism to that in the ambient environment. In contrast, bioaccumulation is accumulation from all sources, both ambient and dietary (Arnot and Gobas, 2006). Biomagnification may be described as the accumulation and transfer of chemicals via the food chain usually resulting in increased concentrations in organisms at higher trophic levels (European Commission, 2003). The biomagnification factor (*BMF*) is the ratio of a chemical's concentration in an organism to that in its diet.

Oxytetracycline (OTC) is a member of the tetracycline family of antibiotics, widely used for therapeutic purposes in humans as well as an antibiotic and growth promoter in animal farming (Holström et al., 2003). It is amongst the most commonly used antibiotics in fish farming in South East Asia (Holström et al., 2003). Previous studies have also demonstrated OTC bioaccumulation in

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0045-6535/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2013.05.070

various organisms including invertebrates and fish (Richardson et al., 2005).

At present, there are growing concerns regarding the ecological/environmental risks associated with OTC exposure. Given its antibacterial properties, development of antibiotic-resistant bacteria in humans, and the environment in general, has been cited as an obvious risk with use (Williams and Brooks, 2012). Antibiotics such as OTC have also been found to be very toxic to algae, the basis of aquatic food chains (Santos et al., 2010). Algal population changes could affect the structure of such chains. There is little evidence for acute toxic effects on fish at environmentally realistic concentrations (Crane et al., 2006). Given a likely entry route to aquatic ecosystems is via agricultural runoff at relatively low concentrations from veterinary use, chronic toxic effects are more likely (Williams and Brooks, 2012).

The number of studies on human risk is relatively small (Kumar et al., 2010). Chronic exposure may result in gastrointestinal irritation and blood composition changes. Acute toxicity is manifested by hepatoxicity (WHO, 2012). Apart from direct therapeutic use, other routes of human exposure are via potable water and ingestion of contaminated food (e.g. fish and shrimp). Concentrations of antibiotics such as OTC in source waters for potable use are typically such that they are not a concern for human health (Boxall, 2004). However herbivorous animals such as fish can bioaccumulate antibiotics that in turn can be transferred to humans through the food chain (Kong et al., 2007). The extent to which this occurs is largely unknown. It has been speculated that this may produce tolerance to pathogens in animal consumers and humans (Boxall et al., 2006), but the direct risk to human health is uncharacterized.

Many factors can affect the bioavailability of OTC in the aquatic food chain. Divalent cations (e.g. Ca^{2+} and Mg^{2+}) in water can bind to OTC and form relatively stable complexes that reduce gastrointestinal absorption efficiency (Sassman and Lee, 2005). Environmental factors such as temperature, pH and light intensity can affect the degradation of OTC in water and sediment (Burhenne et al., 1997). Doi and Stoskopf (2000) found that the compound was more stable at low temperatures (4 °C) while Pouliquen et al. (2007) reported that first order degradation rates of OTC in water increase at least threefold with light exposure (1400 lux) compared to darkness and moreover were pH dependent.

The accumulation of OTC at each trophic level of an aquatic food chain and ultimately the concentration in food for human intake are likely to be affected by these environmental factors, as well as the types of plant and animals involved. In this study, watermeal or swamp algae (*Wolffia globosa* Hartog & Plas.) was employed as the lowest trophic level, bioconcentrating OTC from water. Seven-striped carp (*Probarbus jullieni*) represented the second trophic level, bioaccumulating OTC from water itself and through consumption of contaminated watermeal. Watermeal is a free-floating plant without roots with a wide distribution in rivers across several continents including Asia and North America. The seven-striped carp is a herbivorous fish, found in Thailand, Cambodia, Laos, Malaysia and Vietnam.

The objectives of this study were to investigate the accumulation of OTC in watermeal and seven-striped carp as the first and second trophic levels in an aquatic food chain and evaluate whether OTC can undergo food chain transfer. Finally, derived concentrations of OTC in seven-striped carp were employed to assess the risk involved in human consumption (EPA, 1989). The food chain considered is illustrated in Fig. 1.

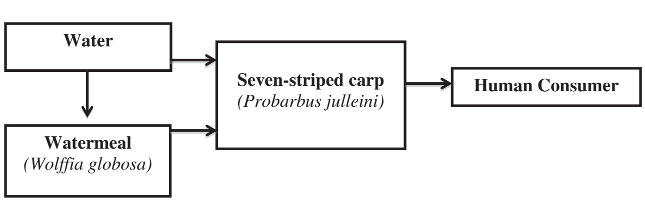

Fig. 1. Aquatic food chain for transfer of OTC to humans.

2. Materials and methods

2.1. Reagents

Oxytetracycline standard (as the hydrochloride) (>98.5% purity) for HPLC analysis was purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany. Commercial grade oxytetracycline hydrochloride used in experiments was obtained from Nova Medicine, Bangkok, Thailand and checked for impurities prior to use. Acetonitrile (Nanograde, Mallinckrodt, KY, USA), methanol (HPLC grade, Fisher, Pittsburgh, PA, USA), oxalic acid (AR, Ajax, Sydney, Australia), sodium EDTA (AR, Ajax, Sydney, Australia) and glass fiber filter papers (GF/C, Whatman, Maidstone, England) were used for extraction of the antibiotics. Solid phase extraction (SPE) tubes (Strata-X Polymeric Reversed Phase (200 mg/6 mL)) were obtained from Phenomenex (Torrance, CA, USA) and conditioned with 2 mL methanol followed by 2 mL of deionized water. Water used in accumulation experiments with fish was dechlorinated tap water [Ca^{2+} = 33.8 mg L^{-1}; Mg^{2+} = 8.8 mg L^{-1}].

2.2. Analysis of OTC in water, plant and fish samples

The concentrations of OTC in plant and fish samples (approximately 10 g) were determined by adding 25 mL of McIlvaine buffer-EDTA solution and followed the method described in Boonsaner and Hawker (2010). Samples were blended with an Ultra-Turrax homogenizer. Then, both sample types were placed in an ultrasonic bath for 2 min and filtered through a glass fiber filter. The filtrate was passed through a conditioned SPE cartridge and eluted with 4 mL of methanol. Resulting solutions containing OTC were analyzed by HPLC (Waters 600, Milford, MA, USA) with Photodiode Array Detection. The analytical column was a C18 column (HiQ Sil C18HS, 4.6 mm id × 150 mm and 0.5 μm particle size) operated at room temperature and the chromatographic conditions were as follows: flow rate 1 mL min^{-1}; mobile phase 95% acetonitrile (v/v), 5% 0.01 M oxalic acid (v/v); injection volume 20 μL with detection at 333 nm. Water samples (100 mL) were passed through a conditioned SPE cartridge, eluted with 2 mL of methanol and analyzed by HPLC using the conditions described above. Method detection limits (MDL) for the analysis of OTC in plants and fish were 0.45 and 0.7 mg kg^{-1} dry weight (dw) and 0.5 mg L^{-1} for water. The mean recoveries from water, plant and fish samples were 97%, 70% and 66%, respectively. All concentrations reported herein account for these recoveries.

2.3. Bioconcentration of OTC by watermeal

Watermeal was purchased from a local market in Nakhon Pathom province, central Thailand. Before commencing the experiments, it was determined to be OTC free.

A preliminary investigation of OTC toxicity was undertaken by placing watermeal in 50, 80 and 100 mg L^{-1} solutions. It was found that at concentrations of more than 50 mg L^{-1} toxic effects occurred, evidenced by the watermeal turning brown, gelatinous and malodorous. Therefore, concentrations of 50 mg L^{-1} and less were chosen for bioconcentration experiments.

Experiments were conducted with three initial test OTC concentrations, nominally 10, 30 and 50 mg L^{-1}. For each concentration, 500 mL of test solution (pH 4.4–4.6) was added to each of 8 clear glass jars (800 mL capacity) followed by 20 g of watermeal. This