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Aqueous and dietary bioaccumulation of antibiotic tetracycline in *D. magna* and its multigenerational transfer

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HIGHLIGHTS

- The overall distribution of tetracycline (TCN) in *D. magna* and its surroundings was demonstrated.
- A higher transfer rate of TCN into *D. magna* was observed in aqueous uptake than dietary uptake.
- A higher sorption capacity of TCN onto green algae than *D. magna* was observed.
- Biomagnification of TCN from algae to daphnid is unlikely to occur.
- Biphasic bioaccumulation tendency of TCN was observed in multigenerational exposure.

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ABSTRACT

The potential bioaccumulation and distribution of antibiotics in non-target organisms have been inadequately studied in spite of their widespread occurrence in aquatic systems. We investigated the ability of tetracycline to bioaccumulate through aqueous and dietary routes in an aquatic organism, the freshwater crustacean *Daphnia magna*. *D. magna* was exposed to algal food (*Pseudokirchneriella subcapitata*) contaminated with tetracycline for dietary uptake. Tetracycline was transferred to *D. magna* more through aqueous uptake than through dietary uptake. The uptake rate constant of tetracycline for *D. magna* was $k_{in,water} = 0.33 \pm 0.045$ via the aqueous route and $k_{in,food} = 0.16 \pm 0.012$ via the dietary route for 1.0 mg L^{-1} tetracycline. Bioconcentration factors of 4.40 ± 0.91 L kg^{-1} and 3.66 ± 0.50 L kg^{-1} for 0.1 and 1.0 mg L^{-1} tetracycline were found for *D. magna*. The biomagnification factor of 0.19 ± 0.04 indicates that magnification of tetracycline through the food web will not occur. The change in the internal concentration of the target compound was also studied for multigenerational (F1-F4) exposure. The internal concentration in *D. magna* showed a decreasing trend with increasing generations except for the parent generation. The bioaccumulation tendency showed a biphasic change in multigenerational exposure.

1. Introduction

Pharmaceutical compounds are one of the most commonly detected environmental pollutants due to the enormous amount of consumption, contribution from numerous sources, and ubiquitous contamination. The widespread occurrence of pharmaceuticals has been demonstrated by a number of monitoring studies [1–4]. The continual release of pharmaceuticals into aquatic environments results in continuous exposure of organisms to the pollutants and consequently induces unexpected detrimental effects [5,6].

Tetracycline, which was chosen as a model compound in this study, is an antibiotic of high priority, considering the potential to enter the environment, usage, and the hazard posed to terrestrial and aquatic organisms [7]. Tetracycline is frequently applied in veterinary medicine and growth promotion; therefore, the consumption of tetracycline for veterinary purposes is higher than for other classes of antibiotics.

Because tetracycline is continuously released into aqueous ecosystems, it can be ingested by aquatic organisms and transferred through the trophic chain. However, little is known about the bioaccumulation of tetracycline and other pharmaceuticals in

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aquatic species, though the research need has been increasingly emphasized [8]. A few studies have reported the uptake and depuration of other pharmaceuticals into non-target species, such as aquatic invertebrates [9–11], fishes [12–15], and plants [16–18]. Furthermore, it is necessary to clarify the uptake route of the target contaminants from an aqueous environment to trophic-level organisms, such as *Daphnia magna*. Because daphnids are the primary consumer of algae and are one of the major food sources for fish and invertebrate predators, they play an important role in the food chain by connecting primary producers and carnivores. Moreover, the daphnia species is an important biological indicator of environmental pollution; thus, a number of toxicity tests using daphnids have been conducted [19]. The transfer of pharmaceuticals through the food chain is also required for a reliable ecological risk assessment.

Aquatic organisms may be exposed to pharmaceuticals throughout their entire lifetimes for numerous generations due to the continuous emission of toxicants into aquatic environments. In a previous study, the multigenerational effects of tetracycline on the reproduction and somatic growth of *D. magna* were identified [20]. The reduction of reproduced neonates resulted in a decrease in the population growth rate, indicating the importance of the multigenerational effect of pharmaceuticals. However, little is known about the bioaccumulation trend of tetracycline in *D. magna* over multiple generations. The change in the internal concentration of toxicants in a multigeneration study has been investigated mainly for metal exposure, such as copper [21] and cadmium [22]. The multigenerational toxicity of pollutants can be related to their bioaccumulation. Guan and Wang [22] reported that the accumulation of cadmium in multigenerationally exposed *D. magna* had a similar pattern to the tolerance that can be partially explained by the metallothionein induction as a defense response. However, it is not clear whether there is a causal link between the accumulation of pollutants and their toxic effects.

The goal of our study was to determine the bioaccumulation of tetracycline in daphnia via various routes and the change of the internal concentration under multigenerational exposure. The uptake and depuration of tetracycline in a freshwater crustacean (*D. magna*) and its surroundings were estimated (Fig. 1). Direct uptake through an aqueous medium and the trophic route through algal food (*Pseudokirchneriella subcapitata*) were compared to establish the food chain transfer. Additionally, we quantified the internal tetracycline content in daphnids exposed throughout four successive generations and various concentrations. The influence of multigenerational exposure of tetracycline on the bioaccumulation of the target compound in *D. magna* was investigated.

2. Methodology

2.1. Reagents and D. magna culture

Tetracycline was purchased from Sigma (St. Louis, MO, USA). HPLC-grade methanol and water were purchased from Fisher Scientific (Pittsburgh, PA, USA). Simatone (Accustandard®, New Haven, CT, USA) was chosen as an internal standard because it eluted within the same chromatographic time window as tetracycline antibiotics, responded well in ESI(+), and did not exhibit noticeable matrix effects [23]. All other reagents used for this study were of analytical grade. Ultrapure water was obtained from a Milli-Q water system (Millipore, Bedford, USA).

A single clone of *D. magna* was used to perform the toxicity test. *D. magna* was obtained from a permanent laboratory culture. The *D. magna* culture procedure and preparation of the culture media followed those in the EPA manual [24]. Cultures were fed daily with a suspension of yeast, trout chow, and Cerophyll® (YCT) mixture, as well as green algae (*P. subcapitata*). The culture medium for *D. magna* was reconstituted with moderately hard water (i.e., $CaSO_4$ 120 mg L^{-1}, $NaHCO_3$ 192 mg L^{-1}, $MgSO_4$ 120 mg L^{-1}, and KCl 8.0 mg L^{-1}) and was renewed three times each week. The culture was conducted at 22 ± 1 °C in a temperature-controlled room maintained with a 16 h light and 8 h dark photo-cycle.

2.2. Aqueous uptake and depuration experiments

The test was initiated by quantifying the tetracycline medium concentration for the precise distribution of tetracycline in the daphnia media before uptake. Concentrations of 1 and 0.1 mg L^{-1} of tetracycline in the media were determined at 0, 2, 4, 8, 12, 24, 36, and 48 h. Uptake and depuration studies were performed using *D. magna* exposed to 1 and 0.1 mg L^{-1} tetracycline. Thirty adult animals (21-day-old) were placed in a glass beaker containing 250 mL of a test medium per each time point. The studies consisted of a 24 h exposure phase followed by a 24 h depuration phase. The sampling time points were 0, 2, 4, 8, 12, 24, 26, 28, 32, 36, and 48 h, and the tests were conducted in duplicate. At each sampling point, the exposure medium was also sampled to determine the remaining concentration of the target compound. After the uptake phase, the organisms were transferred into a medium with no tetracycline for a depuration test. The same time points were used in the depuration phase as in the uptake phase. Once removed from the beaker, the organisms were rinsed in deionized water to remove any tetracycline residue from the surface. Thirty organisms per replicate were placed on a paper filter with a pipette to remove excess water from outside the carapace. Daphnids were transferred into a microtube and weighed using an ultra-micro balance (model DRAGON 204, Mettler-Toledo Group). After measuring the wet body weight, the daphnids were immediately frozen with liquid nitrogen and kept at −80 °C until further experiments were conducted.

For the extraction, the organisms were homogenized using a Pellet Pestle motor (Kontes, Vineland, NJ, USA). To 30 organisms, 500 μL of methanol containing 0.2% formic acid and 10 μL of 100 mM EDTA-Na_2 were added and homogenized for 3 min. The samples were then filtered using a 0.22 μm nylon syringe filter (Advantec MFS, Dublin, CA, USA), and analyzed. The exposure solutions were also sampled and filtered with 0.22 μm nylon syringe filters (Advantec MFS, Dublin, CA, USA) before analysis.

2.3. Dietary uptake into D. magna through algal food

The aqueous and dietary uptake tests were performed separately (Fig. 1A). The uptake of tetracycline via algal food by *D. magna* was evaluated using sorption kinetics of tetracycline to *P. subcapitata* and its uptake by daphnid. The test of the sorption of tetracycline to algae was determined using the method of Jeon et al. [25]. Algae (3×10^7 cells/mL) were exposed to the target compound at concentrations of 0.01, 0.1, and 1 mg L^{-1}. A culture of 50 mL algal solution was conducted in an incubator at 22 ± 1 °C and 100 rpm (revolutions per minute). The concentration of aqueous tetracycline was determined at 0, 2, 4, 8, 12, 24, 36, and 48 h. Samples at each time point were filtered through a 0.22 μm nylon syringe filter and analyzed. The rate of biosorption of tetracycline was evaluated using the following kinetic model (1):

$$f(t) = f_{water} + a(1 - e^{-bt}) \quad (1)$$

where t is the exposure time (h), $f(t)$ is the fraction of the target compound remaining in the solution at time t, f_{water} is the fraction at a steady state, and a and b are coefficients. The partition coefficient on algae ($K_{d,algae}$) was defined as the ratio of tetracycline in the sorbent (C_{algae}) and in water (C_{water}) at equilibrium (2). It was