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Toxicity of the veterinary sulfonamide antibiotic sulfamonomethoxine to five aquatic organisms

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ABSTRACT

The purpose of this study was to investigate the acute and chronic toxicity of sulfamonomethoxine (SMM) to aquatic organisms to evaluate its impact at different trophic levels in the ecosystem. Regarding the growth inhibition of microalgae, SMM exhibited 72-h median effective concentration (EC_{50}) values of 5.9 mg L^{-1} for freshwater *Chlorella vulgaris* and 9.7 mg L^{-1} for marine *Isochrysis galbana*. In a study on the cladocerans, SMM exhibited acute toxicity and 48-h median lethal concentrations of 48 mg L^{-1} for *Daphnia magna* and 283 mg L^{-1} for *D. similis*. An examination of chronic toxicity revealed that SMM inhibited the brook production of the cladocerans and exhibited 21-day EC_{50} values of 14.9 mg L^{-1} for *D. magna* and 41.9 mg L^{-1} for *D. similis*. This study investigated the potentially adverse effects of SMM on aquatic organisms and revealed that microalgae exhibited higher sensitivity to SMM than cladocerans did. The residue of SMM in water is recommended to be carefully evaluated to reduce ecological impacts after applied to cultured animals.

1. Introduction

Antibiotics play a vital role in treating and preventing diseases among animals in the modern livestock and aquacultural industries. Antibiotics are usually administered to animals through feed; however, this practice often results in the release of antibiotic residue from the medicated feed, animal feces, and even water runoff from manure-treated farmland that contaminates surrounding surface water (Nikolaou et al., 2007). This residue may exert a direct toxic effect on microflora and microfauna (Lai et al., 2009), facilitate the development of antibiotic-resistant bacterial populations (Kümmerer, 2009), and transfer antibiotic resistance to pathogenic microbes that affect humans (Baran et al., 2011).

Sulfonamide antibiotics (SAs) are widely used to medicate farm animals and constitute a high proportion of the total global usage of antibiotics today (Baran et al., 2011). SAs are frequently excreted in the urine and feces of treated animals as parent compounds or metabolites that are easily transferable from contaminated sites to surrounding water because they are not easily sorbed by soils and sediments (Baran et al., 2011; Sukul and Spiteller, 2006). SA residue has been detected in fish

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ponds (Dietze et al., 2005; Le and Munekage, 2004) and their effluents and downstream water (García-Galán et al., 2010; Lin et al., 2008; Luo et al., 2011; Tamtam et al., 2008).

Sulfamonomethoxine (SMM) is one of the broad-spectrum SAs commonly used in aquaculture (Arthur et al., 2000) that has been detected in the water and effluents of aquaculture ponds (Lin et al., 2008), in sewage sludge (wastewater) released by treatment plants (Okuda et al., 2009), and in manure produced by farm animals (Zhao et al., 2010). An SMM concentration as high as 100 $\mu g L^{-1}$ was detected in sewage sludge (Okuda et al., 2009).

Despite the extensive use of SMM in aquaculture and its wide contamination of surface water, information on the toxicity of SMM to aquatic life is insufficient. Furthermore, discrepancies in the toxicity of SAs to aquatic organisms have been reported; some studies have reported significant toxic effects (De Liguoro et al., 2010; Eguchi et al., 2004; Isidori et al., 2005), whereas other studies have not (Lin et al., 1993). These discrepancies might be due to differences in the sensitivity of the tested organisms and criteria used for defining the toxic effects (Baran et al., 2011).

The purpose of this study was to conduct bioassays to investigate the acute and chronic toxicities of individual SMM to aquatic organisms at various trophic levels in the ecosystem. Based on the results obtained, the environmental hazards of SMM were evaluated, and the aspects of aquaculture effluent management concerning the antibiotic are discussed.

2. Materials and methods

2.1. Antibiotic

SMM [4-amino-N-(6-methoxy-4-pyrimidinyl) benzenesulfonamide, CAS 1220-83-3] with a purity of 98% was purchased from Sigma–Aldrich (St. Louis, MO, USA) and dissolved in a 0.03 M NaOH solution to create a stock solution with a concentration of 5000 $mg L^{-1}$. Tap water was distilled and then deionized using the Milli-Q Plus system (Bedford, MA, USA). This deionized water was used in preparing the NaOH solution as well as the stock and test SMM solutions. All chemicals used in this study were high-performance liquid chromatography (HPLC) grade.

2.2. Organisms

Five species of aquatic organism were examined: a freshwater microalga (*Chlorella vulgaris*), a marine microalga (*Isochrysis galbana*), two freshwater cladocerans (*Daphnia similis* and *Daphnia magna*), and a freshwater medaka fish (*Oryzias latipes*). The two microalgae have commonly been used as indicator species for primary producers in aquatic food chains and ecosystems (Lai et al., 2009). Because these microalgae have high protein and fatty acid contents, they are also used as feed in larval culture of aquatic organisms (Spolaore et al., 2006). The cladocerans are an effective bioindicator of toxicity because of their high sensitivity and short reproductive cycle (OECD 202, 2004). *O. latipes* is a common freshwater fish that is used in acute and chronic toxicity tests (OECD 203, 1992).

2.3. Stock cultures

2.3.1. Algae

C. vulgaris was obtained from the Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan in 2007. *I. galbana* was obtained from the Tungkang Biotechnology Research Center, Fisheries Research Institute, Pingtung, Taiwan. In the laboratory, both algae were cultured in a 1.5-L conical flask containing 1000 mL of culture media. The media for *C. vulgaris* were prepared according to guidelines of the Organization for Economic Cooperation and Development (OECD) (OECD 201, 2006), and the Walne medium (Walne, 1974), which was used for *I. galbana*, was prepared using seawater at a salinity of 34‰. The seawater was prepared by mixing artificial sea salt (TAAM, Camarillo, CA, USA) in the deionized water.

The culture media were UV-irradiated for 30 min and then filtered with a 0.2-μm filter before use. After inoculation, the cultures were gently aerated at a temperature of $25 \pm 1\,^{\circ}C$ under continuous illumination at 6 klx. The pH values were 7.2–7.8 for *C. vulgaris* and 6.8–7.0 for *I. galbana*. The cultures were renewed weekly.

2.3.2. Cladocerans

D. magna was obtained from the Environmental Analysis Laboratory of the Environmental Protection Administration, Taoyuan, Taiwan. *D. similis* was procured from the Freshwater Aquaculture Research Center, Fisheries Research Institute, Chupei, Taiwan. The cladocerans were separately cultured in 10-L glass jars containing 8 L of dechlorinated tap water (hardness 146 $mg L^{-1}$, conductivity 534–585 $\mu S\,cm^{-1}$) at a constant temperature of $25 \pm 1\,^{\circ}C$ during a 16-h light (L):8-h dark (D) period under 2 klx of white fluorescent light in accordance with OECD guidelines (OECD 202, 2004; OECD 203, 1992). The culture water was renewed and the glass jars were replaced once per week. The cladocerans were fed with the green alga *C. vulgaris* once per day at an average density of approximately 3×10^5 cells mL^{-1}.

2.3.3. Medaka fish

O. latipes was obtained from the Freshwater Bioresource Center, National Chiayi University, Taiwan. The fish were kept in 20-L aquaria containing 15 L of the dechlorinated tap water. The culture temperature, light intensity, and light periods were similar to those used for the daphnids. The fish were fed with freshly hatched *Artemia nauplii* and artificial feed (Trifish BP, Omega, Kaohsiung, Taiwan) once per day. Feeding was stopped 1 day before the test began.

2.3.4. Acclimation

In this study, the culture water and media used in the stock cultures of the test organisms were the same as those used in the solutions for the toxicity tests. In addition, the culture conditions (temperature, light intensity, and light–dark periods) applied to the stock cultures were similar to those applied in the toxicity tests. Therefore, the organisms in the stock cultures were considered to be acclimated and were used directly in the tests.