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Ecotoxicity of the two veterinarian antibiotics ceftiofur and cefapirin before and after photo-transformation

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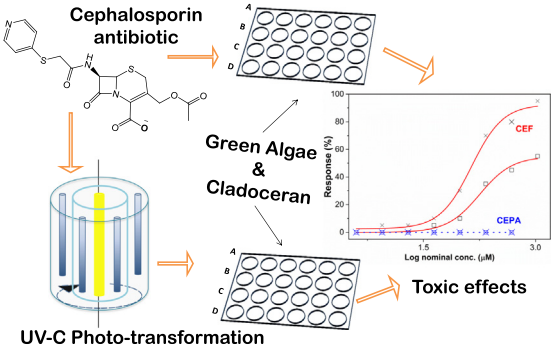
HIGHLIGHTS

- Cephalosporins exhibited instability in green algae and cladocera media.
- Effects of veterinary antibiotics on green algae and cladocera are shown.
- Ceftiofur effective concentrations to *D. magna* are at mg L^{-1} range.
- UV-C radiation at 254 nm increased cefapirin's acute toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

The release of antibiotics into the environment may lead to deleterious effects in non-target organisms as well as pressure in antimicrobial resistance acquirement. Ceftiofur (CEF) and cefapirin (CEPA) are veterinary cephalosporins used for recurrent and economically relevant infections. Both antibiotics have been detected in aquatic environments and their fate during drinking water processing is still unknown. This work investigated the acute and chronic toxicities of CEF and CEPA towards aquatic organisms including stability tests. Complementary, the effects of water disinfection radiation (UV-C, 254 nm) on ecotoxicological responses were studied. CEF and CEPA have significant decay during *Daphnia magna* tests, portraying half-lives ($t_{1/2}$) of 49 and 53 h, respectively. During tests with green algae (*Scenedesmus* spec.), CEPA was more instable ($t_{1/2}$ 88 h) than CEF ($t_{1/2}$ 267 h). CEF and its presumable hydrolysis products induced deleterious effects in *Daphnia magna* (48 h EC_{50} 139, LC_{50} 179 in µM), which was not observed with *Scenedesmus* spec. (72 h NOAEC 82.5 ± 2.5 µM). In the case of CEPA, no toxic effects were observed in either test (48 h EC-LC_{50} > 510 and 72 h NOAEC 57 ± 6, in µM). Photolysis of CEPA resulted in toxic products, which were effective for the cladoceran but not for the green algae. On the other hand, the different radiation doses studied did not affect CEF ecotoxicity. This investigation illustrates the importance of cephalosporin hydrolysis during standard toxicity tests. Furthermore, the potential formation of species-specific toxic compounds during water processing is demonstrated, highlighting the need of further assessing toxicity of both cephalosporins and their transformation products.

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1. Introduction

The behavior of cephalosporin antibiotics in the aquatic environment is very dynamic and complex (Yamana and Tsuji, 1976; Gilbertson et al., 1990; Kümmerer, 2009; Wang and Lin, 2012). Concern about cephalosporins involve possible undesirable effects on non-target organisms (Wang and Lin, 2012; Chen and Guo, 2012; Magdaleno et al., 2015; Li and Lin, 2015), as well as the occurrence, spread, and increase of antibiotic-resistant bacteria (EMA, 2012, 2015; WHO, 2014). Zhang et al. (2010) reported on teratogenic effects of cefazolin and cefazedone in zebrafish embryo tests. Cefradine and its photolytic products presented chronic toxicity to freshwater algae (Chen and Guo, 2012). Representatives of these antibiotics have been detected in aqueous matrices at concentrations ranging from ng L^{-1} to μg L^{-1} (Cha et al., 2006; Lin et al., 2008; Tamura et al., 2017; Biel-Maeso et al., 2018). Recently, traces of ceftiofur (CEF) were detected in two locations in Japan (Tamura et al., 2017) and in coastal waters of Spain (Biel-Maeso et al., 2018). The measured concentrations by Tamura et al. (2017) were 1.6 ng L^{-1} (WWTP effluent in Kyoto) and 0.9 ng L^{-1} (sept tank effluent in Tokushima). Likewise, ceftiofur was detected in river discharge areas (Sancti-Petri Channel, Cadiz Bay), with concentrations ranging from not detected to 1.7 ng L^{-1} (Biel-Maeso et al., 2018).

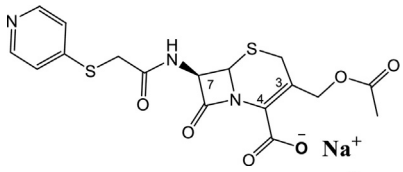
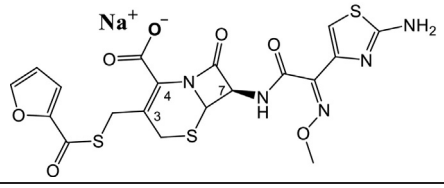
Average detected concentrations of cefapirin (CEPA) were 5 and 9 ng L^{-1}, obtained in hospital wastewater of Taiwan (Lin et al., 2008) and in an agriculturally influenced river in USA (Cha et al., 2006), respectively. However, when compared to other antibiotics, the frequency of reported cephalosporins' occurrence in the environment can be considered low (Cha et al., 2006; Junker et al., 2006; Tamura et al., 2017). According to Junker et al. (2006), this is mainly due to analytical limitations and to transformations of the parental structure. However, considering the wide use and importance of this antibiotic group, the knowledge of cephalosporins' environmental fate is insufficient (Eguchi et al., 2004).

In human medicine, cephalosporins are primarily used as second- or third-line therapy (BPAC, 2011). However, in veterinary treatments cephalosporins are intensively used (Gilbertson et al., 1990; Salmon et al., 1996; Eguchi et al., 2004; WHO, 2014). CEF and CEPA are applied especially for the treatment of common and recurrent animal diseases (Gilbertson et al., 1990; Salmon et al., 1996; Sadeghi-Sefidmazgi et al., 2011).CEF is used for treatment of a large range of respiratory, urinal and dermatologic diseases (Salmon et al., 1996). Likewise, the intramammary infection mastitis, which leads to economic impacts in the milk industry, is usually treated by CEPA (Sadeghi-Sefidmazgi et al., 2011). These drugs possess as core structure of the 7-aminocephalosporanic acid, known as cephem ring, as well as substituents at C-3 and C-7 position (Table 1), which are responsible for both the pharmacokinetic and antibacterial effects (Salmon et al., 1996). This antibiotic group has a broad activity spectrum presenting high efficiency towards both Gram-positive and Gram-negative bacteria by interrupting bacterial cell walls (EMA, 2012, 2015).

Several data suggested that abiotic transformations are more significant than biodegradation for the environmental degradation of cephalosporins in surface water and wastewater (Gilbertson et al., 1990; Jiang et al., 2010; Li et al., 2011). Hydrolysis is a relevant mitigation process due to the expected fast degradation of cephalosporins' side-chains and the beta lactam ring cleavage (Yamana and Tsuji, 1976; Mitchell et al., 2014). Wang and Lin (2012) studied photolysis of cephalosporins under simulated sunlight radiation and reported a significant increase of acute toxicity towards the luminescent bacteria *Vibrio fischeri*. Similarly, cefradine and its photo-degradation products presented adverse effects to chlorophyceae and cyanophyceae (Chen and Guo, 2012). Besides the importance and increasing use of ultraviolet radiation (UV-C) for water disinfection and wastewater purification, few data are available with regard to cephalosporins' fate during drinking water processes (Kim and Tanaka, 2009). In fact, even basic data reporting on ecotoxicity of cephalosporins and their transformation products is lacking (Zhang et al., 2010; Liu et al., 2011; Wang and Lin, 2012; Chen and Guo, 2012; Rahul et al., 2015; Magdaleno et al., 2015). Although little explored, the mode of action of cephalosporins and their byproducts in non-target organisms may be related to their toxic substituents (Zhang et al., 2010; Wang and Lin, 2012) and to formation of reactive oxygen species (Rahul et al., 2015).This lack of knowledge requires further investigation, since the presence of such compounds in aquatic systems cannot be neglected.

Therefore, the present study investigated the acute and chronic toxicities of CEF and CEPA. Complementary, the effects of different UV-C doses on CEF and CEPA ecotoxicity were assessed using two standardized and well established toxicity essays. Acute tests were carried out using the cladoceran *Daphnia magna* (OECD, 2004; Zimmermann et al., 2017), while chronic tests were performed with the green algae *Scenedesmus* spec. (Eisentraeger et al., 2003; OECD, 2011; ISO 8692, 2012). Knowing that cephalosporins' hydrolysis may interfere in exposure concentrations and result interpretations, the present study followed the recommendations for test validations presented in the respective OECD standard guidelines (OECD, 2000, 2004, 2011).

Table 1
Description of investigated cephalosporins.

Name Abbreviation	Chemical properties[a]	
Cefapirin CEPA		$C_{17}H_{16}N_3NaO_6S_2$ MW = 445.44 g mol^{-1} pK_{a1} = 2.74 ($COOH \rightarrow COO^-$)[b] pK_{a2} = 5.13 ($NH^+ \rightarrow N$)[b] k^{app} = 0.0116 mJ cm^{-2}[c]
Ceftiofur CEF		$C_{19}H_{16}N_5NaO_7S_3$ MW = 545.54 g mol^{-1} pK_{a1} = 2.68 ($COOH \rightarrow COO^-$)[b] pK_{a2} = 3.53 ($NH^+ \rightarrow N$) k^{app} = 0.0098 mJ cm^{-2}[c]

[a] ChemAxon (2017).
[b] Ribeiro and Schmidt (2017).
[c] Pseudo-first order degradation rate (k^{app}), determined during photolytic transformation at pH = 7 and 254 nm (Ribeiro, 2017).