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Aquatic toxicity of four veterinary drugs commonly applied in fish farming and animal husbandry

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HIGHLIGHTS

- Ecotoxicity of four veterinary drugs to four aquatic organisms was evaluated.
- New data about their ecotoxicological potential has been presented.
- All the ecotoxicological tests were supported by chemical analyses.
- DOR was found to be highly toxic toward *Daphnia magna.*

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ABSTRACT

Doramectin (DOR), metronidazole (MET), florfenicol (FLO), and oxytetracycline (OXT) are among the most widely used veterinary drugs in animal husbandry or in aquaculture. Contamination of the environment by these pharmaceuticals has given cause for concern in recent years. Even though their toxicity has been thoroughly analyzed, knowledge of their ecotoxicity is still limited. We investigated their aquatic toxicity using tests with marine bacteria (*Vibrio fischeri*), green algae (*Scenedesmus vacuolatus*), duckweed (*Lemna minor*) and crustaceans (*Daphnia magna*). All the ecotoxicological tests were supported by chemical analyses to confirm the exposure concentrations of the pharmaceuticals used in the toxicity experiments, since deviations from the nominal concentration can result in underestimation of biological effects. It was found that OXT and FLO have a stronger adverse effect on duckweed (EC_{50} = 3.26 and 2.96 mg L^{-1} respectively) and green algae (EC_{50} = 40.4 and 18.0 mg L^{-1}) than on bacteria (EC_{50} = 108 and 29.4 mg L^{-1}) and crustaceans (EC_{50} = 114 and 337 mg L^{-1}), whereas MET did not exhibit any adverse effect in the tested concentration range. For DOR a very low EC_{50} of 6.37 × 10^{-5} mg L^{-1} towards *D. magna* was determined, which is five orders of magnitude lower than values known for the toxic reference compound $K_2Cr_2O_7$. Our data show the strong influence of certain veterinary drugs on aquatic organisms and contribute to a sound assessment of the environmental hazards posed by commonly used pharmaceuticals.

1. Introduction

Large quantities of veterinary pharmaceuticals (VPs) are in use worldwide. As animals do not completely metabolize these compounds, a large proportion of them are excreted unchanged in feces and urine. Therefore, both the drugs and their metabolites are released into the environment, either directly from aquaculture and by grazing animals, or indirectly during manure spreading (Reemtsma and Jekel, 2006).

Of the various pharmaceuticals commonly used in veterinary medicine, special attention has been paid to four of them in the present work: doramectin, metronidazole, florfenicol and oxytetracycline, which differ in their activity and physicochemical properties (Table 1).

OXT, FLO and MET are antibiotics and have a similar mode of action at the DNA/RNA-level. OXT is commonly used because of its broad-spectrum efficacy in the treatment of infections caused by Gram-positive and Gram-negative bacteria, mycoplasma and large viruses. It inhibits protein synthesis by preventing the association of aminoacyl-tRNA with bacterial ribosomes (Reemtsma and Jekel, 2006). FLO is a fluorinated derivative of thiamphenicol, inhibits transpeptidation in the bacterial protein synthesis, and is effective

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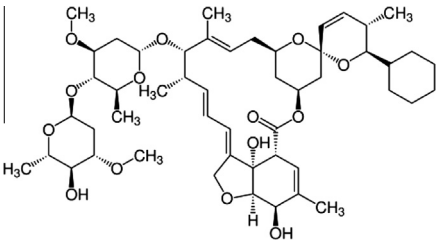
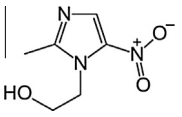
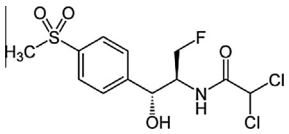
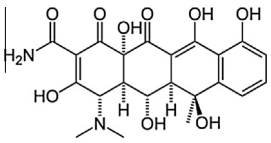
Table 1
Structures and physicochemical properties of the investigated veterinary drugs.

Substance (Abbreviation) [CAS]	Structure	M.w. (g mol^{-1})	pK_{a1}	pK_{a2}	pK_{a3}	Log P	Water solubility (mg L^{-1})
Doramectin (DOR) [117704-25-3]		899.1	12.4	–	–	4.44	0.025
Metronidazole (MET) [443-48-1]		171.2	2.4	–	–	−0.1	10000
Florfenicol (FLO) [73231-34-2]		358.2	9.3	–	–	−0.12	1320
Oxytetracycline (OXT) [96310-42-8]		460.4	3.3	3.7	9.1	−1.22	>100000 (HCl salt)

against many Gram-negative and Gram-positive bacteria (Christensen et al., 2006). The nitroimidazole MET is an antibiotic effective against anaerobic bacteria, protozoans and certain parasites. It acts by entering bacterial and protozoan cells and interfering with DNA (Lanzky and Halling-Sørensen, 1997). DOR is an antiparasitic drug which is a one of the most popular compound for curing anthelmintic disease. DOR binds to receptors that increase membrane permeability to chloride ions. This inhibits the electrical activity of nerve cells in nematodes and muscle cells in arthropods, paralyzing and ultimately killing the parasites (Horvat et al., 2012; Lumaret et al., 2012). In many countries these pharmaceuticals are registered as medical premixes so they can be used in feedstuffs for the treatment or prevention of animal diseases. For example the usage of OXT in the UK in 2000 was 8.5 t and in the US, just in aquacultures in 2003, was 15 t (Sarmah et al., 2006; Schmidt et al., 2007). Furthermore, in accordance to the IMS Health Market Prognosis (2012) the global pharmaceutical market has grown twice during the last decade. However the real amounts of drugs entering the environment can be much more higher due to lack of control with type and amounts of pharmaceuticals usage in developing countries e.g. India, Thailand, Indonesia (Sarmah et al., 2006). The relatively low cost and the broad spectrum of activity of these pharmaceuticals means they are very commonly used not only in animal husbandry (DOR, MET, FLO, OXT) but also in aquaculture (MET, FLO, OXT) (Christensen et al., 2006; Ferreira et al., 2007; Lai et al., 2009; Lumaret et al., 2012; Horvat et al., 2012).

It must be pointed out that the veterinary medicines used in aquaculture are commonly administered as a medicated feed mainly as a bath formulation (Hekoten et al., 1995; Boxall, 2010). Hence a considerable proportion of these drugs administered in an intensive fish farm was found to have been released into the aquatic environment via urinary and fecal excretions and in unconsumed medicated food (Hekoten et al. 1995). For example, Ferreira et al. (2007) highlighted that when OXT was administrated orally, fish took up only about 10–30% of the total amount administered, while 70–90% of it entered the environment and was available for distribution to other compartments.

Disregarding the different routes by which these pharmaceuticals enter the environment, their presence in its different compartments has already been determined (Kolpin et al., 2002; Kay et al., 2005). Despite their quite low concentrations in environmental samples at the μg L^{-1} or ng L^{-1} level (MET – 30 ng L^{-1}, OXT – 340 μg L^{-1}, FLO – 2.4 μg L^{-1}) (Kolpin et al., 2002; da Silva et al., 2011; Wei et al., 2012) and their different stabilities in the environment (FLO is resistant to abiotic degradation but easily biodegradable; OXT is susceptible to photodegradation; MET cannot be biodegraded, photodegradation under UV light is also less effective; DOR is quite susceptible to both biodegradation or photodegradation), they are continuously being released into ecosystems (Jacobsen and Berglind, 1988; Oka et al., 1989; Lunestad, 1992; Pfizer Inc., 1996; Doi and Stoskopf, 2000). Consequently, these compounds may be considered pseudo-persistent. Therefore, the kind of exposure organisms may be subjected to will resemble that of traditional pollutants (e.g. pesticides, detergents).

This may result in adverse ecological effects, including, for example, the development of resistant bacterial populations or direct toxicity to microflora and microfauna. Since these compounds (like FLO and MET) are polar pollutants with quite a low sorption potential and/or are directly introduced into aquatic environment (like OXT), it can be assumed that aquatic organisms may be the species most endangered by the presence of pharmaceuticals used in aquaculture and animal husbandry. Kolar and Kožuh Eržen (2006) reported that DOR is excreted mainly with the feces: almost 98% of the drug is excreted as the non-metabolized