AQUATIC TOXICITY OF FOUR SELECTED VETERINARY DRUGS COMMONLY APPLIED IN FISH FARMING AND ANIMAL HUSBANDRY

BIAŁK-BIELIŃSKA A.*, MASZKOWSKA J.*, KOŁODZIEJSKA M.*, STEUDTE S.*, KUMIRSKA J.*, STEPNOWSKI P.* and STOLTE S.**

*Department of Environmental Analysis, Institute of Environmental Protection and Human Health, Faculty of Chemistry, University of Gdańsk, ul. Sobieskiego 18, 80-952 Gdańsk, Poland
**UFT - Center for Environmental Research and Sustainable Technology, University of Bremen, Leobener Straße, D-28359 Bremen, Germany
e-mail: abialk@chem.univ.gda.pl

ABSTRACT
Oxytetracycline (OXT), florfenicol (FLO), doramectin (DOR) and metronidazole (MET) belong to most widely used veterinary drugs in animal husbandry and in aquacultures. The contamination of the environment by these pharmaceuticals has raised concern in recent years. Even though their toxicological analysis has been thoroughly performed, knowledge of their (eco)toxicity is still very limited. We investigated the aquatic toxicity using tests with luminescent marine bacteria (Vibrio fischeri), limnic unicellular green algae (Scenedesmus vacuolatus), duckweed (Lemna minor) and crustacean (Daphnia magna). All ecotoxicological tests were supported with chemical analysis to confirm the exposure concentration of the pharmaceuticals used in the toxicity experiments. 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) as well as green algae (EC$_{50}$=40.4 and 18.0 mg L$^{-1}$) rather than to bacteria (EC$_{50}$=101 and 29.4 mg L$^{-1}$) and crustacean (EC$_{50}$=114 and 337 mg L$^{-1}$), whereas MET did not cause any adverse effect in the tested concentration range. For DOR a very low EC$_{50}$ value of 6.25x10$^{-5}$ mg L$^{-1}$ towards Daphnia magna was determined what is three orders of magnitude lower than values known for the toxic reference compound K$_2$Cr$_2$O$_7$ (range 0.6 – 2.1 mg L$^{-1}$). Our data reveals the strong influence of some veterinary drugs on aquatic organism and contributes to a sound assessment of environmental hazards related to commonly used pharmaceuticals.

KEYWORDS: ecotoxicity, oxytetracycline, florfenicol, doramecin, metronidazole, aquatic organisms

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 unmetabolized drugs and their metabolites are released into the environment, either directly in aquaculture and by grazing animals, or indirectly during manure spreading. Disregarding the different routes by which these pharmaceuticals enter the environment, their presence in its different compartments has already been determined. However, knowledge about their effect on environment and aquatic organisms is still very limited. Therefore, the aim of our study was to enrich our hitherto limited knowledge of the potentially deleterious effects on the environment of commonly used VPCs in aquacultures or animal husbandry (Boxall, 2010).
2. METHODOLOGY
A battery of (eco)toxicological tests comprising organisms of different trophic levels and complexity: luminescent marine bacteria (Vibrio fischeri), limnic unicellular green algae (Scenedesmus vacuolatus), duckweed (Lemma minor) and crustaceans (Daphnia magna) was used. All test were performed according to internationally accepted test guidelines (e.g. OECD 202, ISO 8692, ISO 6341) or on specific (e.g. national) testing guidelines (e.g. DIN 38412-L34). Dose-response curve parameters and plots were obtained using the drift package (version 0.05-95) for the R language and environment for statistical computing (www.r-project.org) (R Development Core Team, 2005).
In order to determine the soluble fraction of the investigated compound in biological media HPLC analysis was performed using different conditions depending on the analyte. The stock solution concentrations of DOR, MET, FLO and OXT in media solutions used in four ecotoxicity tests were estimated by fitting chromatographic peak areas to the calibration curve.

3. RESULTS
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$_{50}$=101 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 Daphnia magna was determined. The results show that the concentration of the soluble fraction of MET, FLO and OXT in biological media did not differ from the nominal concentration of these drugs prepared in media solution.

4. CONCLUSIONS
As a result of such a comparative (eco)toxicological analysis, the potential effects of four selected veterinary drugs, most commonly used in livestock or in aquacultures, on aquatic organisms was defined. DOR was found to be highly toxic toward Daphnia magna. Even if environmental concentrations and “local hot spots” during the application of these pharmaceuticals are still much lower that the EC$_{50}$s of these pharmaceuticals vis-à-vis certain organisms, it must always be borne in mind that these drugs occur in natural media usually together with others of the same family or type. Therefore, mixture effects need to be considered, as in such situations toxic thresholds could already be reached. Despite these reservations, the ecotoxicological test battery used in this study is a suitable tool for compiling a comprehensive hazard profile for environmentally relevant contaminants.

Acknowledgments: Financial support was provided by Polish National Science Centre under grant DEC-2011/03/B/NZ8/03009 and by the German Academic Exchange Service (DAAD).

REFERENCES