Investigating acute and chronic mixture effects of pharmaceuticals towards *Daphnia magna*

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Pharmaceuticals are compounds with biological potency used to cure diseases and ameliorate human health. In the environment however, pharmaceuticals may retain some or all of their properties and therefore have the potential to cause adverse effects to unintendently exposed organisms (Corcoran et al., 2010; Fong and Ford, 2013). Pharmaceuticals are therefore considered as contaminants of emerging concern. Pharmaceuticals directly enter the environment after disposal of unused pharmaceutical products. Indirectly they are released through the disposal of the treated urban wastewater in surface waters or through reuse in agricultural practices. The presence of pharmaceuticals in treated wastwaters is due to their incomplete removal after the "traditional" biological treatment at wastewater treatment plants. In fact, numerous pharmaceuticals end up in water bodies and soils as complex mixtures of parent compounds, metabolites and transformation products (Michael et al., 2014; Vasquez et al., 2014). Despite the great number of the pharmaceuticals found in the environment, evidence mounts of the role of the significance of the most abundant pharmaceuticals in determining whole mixture effects (Backhaus and Karlsson, 2014).

In the present study a selection of twelve highly consumed and of environmental concern pharmaceuticals were investigated namely, atenolol, metoprolol, propranolol, sotalol, diclofenac, ibuprofen, erythromycin, ofloxacin, sulfamethoxazole, trimethoprim and carbamazepine. In acute experiments, compounds were grouped according to their therapeutic group and evaluated in combination with the other groups. Treated wastewater spiked with pharmaceuticals were also evaluated, so as to increase the understanding on potential complex matrix effects. Acute effects on the immobilization at 24 and 48 h towards *D. magna* indicated mainly antagonistic behaviour at concentrations in the low mg/L range (5-15 mg/L). A fractional factorial design was implemented for the chronic experiments using a two-level orthogonal design. A 12-run Plackett and Burman design was used (Box et al., 2005). Synergistic effects were demonstrated at chronic exposures of 21-day on both reproduction potential and immobilization (0.5 and 5 μg/L). Of the eleven pharmaceuticals erythromycin and propranolol were found to be the compounds driving the main effect of the mixture.

This work provides an approach for investigating effects of mixture of numerous compounds and reducing the total number of experiments. It was shown that antagonistic effects were mainly observed after acute exposures; whereas this trend was inversed at chronic ones. This finding reinforces the general opinion that contaminants of emerging concern should be evaluated for their chronic effects.

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