Ecotoxicological assessment of four pharmaceuticals compounds through acute toxicity tests

Avaliação ecotoxicológica de quatro diferentes fármacos usando testes de toxicidade aguda

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Abstract

In this study, we evaluated acute toxicity of four different pharmaceutical compounds: 17 α-ethinylestradiol (EE2), fluoxetine, diclofenac and ibuprofen to *Daphnia similis*. The average values of EC50 were 1.63 mg/L to the 17α-ethinylestradiol (EE2), 4.41 mg/L to the fluoxetine, 46.0 mg/L to the diclofenac and 97.0 mg/L to the ibuprofen. The effects of these drugs, in particular those caused to aquatic biota, still unknown especially at low concentrations in a range from ng/L up to mg/L.

Keywords: Estrogens. Serotonin Uptake Inhibitors. Anti-Inflammatory Agents. Analgesics. Toxicity.

Resumo

Este estudo avaliou a toxicidade aguda de quatro diferentes fármacos: 17 α-ethinylestradiol (EE2), fluoxetina, diclofenaco e ibuprofeno à *Daphnia similis*. Os valores médios de CE50 foram de 1,63 mg/L para 17 α-ethinylestradiol (EE2), 4,41 mg/L para fluoxetina, 46,0 mg/L para diclofenaco e 97,0 mg/L para ibuprofeno. Os efeitos desses fármacos, sobretudo à biota aquática, ainda são pouco conhecidos especialmente em baixas concentrações na ordem de ng/L a mg/L.

Palavras-chave: Estrogênios. Inibidores de Captação de Serotonina. Anti-Inflamatórios. Analgésicos. Toxicidade.

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INTRODUCTION

Since 1990, several works has been monitoring the presence of pharmaceutical compounds on environment matrices[1,2]. These potentially toxic substances has often been found in effluents of sewage treatment plants, water supply and other environmental matrices such as soil, sediment and natural waters, in concentrations ranging from ng/L to mg/L[2]. Human pharmaceuticals get in aquatic environments mainly via domestic use[1], either on their regular form (unused drugs disposed of down drains) or partially metabolized (excreted compounds)[3]. Recent research in different countries, has demonstrated the presence of multiple classes of pharmaceuticals, such as endocrine disrupting chemicals, psychiatric drugs and nonsteroidal anti-inflammatory drugs in municipal wastewater[4,5,6]. In Brazil, was reported by Stumpf[2] the presence of hormones and anti-inflammatory in sewage, treated wastewater and water from rivers in the state of Rio de Janeiro[2].

The 17 α-ethinylestradiol (EE2) is a synthetic female sex hormone, which is present in contraceptive pills, used both as birth control substance and in hormone replacement therapy[7]. Just in the EU, the production of this compound is around hundreds of kilograms per year[5]. Furthermore, this compound appears to be, extremely persistent in the environment[5]. Fluoxetine is a selective serotonin reuptake inhibitor which is used as active ingredient in anti-depressant drugs widely prescribed currently[8].

Diclofenac is used in human healthcare as an analgesic, antiarthritic and antirheumatic. This compound belongs to the group of the nonsteroidal drugs, which has been used worldwide. Ibuprofen is a nonsteroidal anti-inflammatory, which has been extensively used as an analgesic and antipyretic, also used in the treatment of rheumatic disorders, pain, and fever[2,9]. Although the presence of pharmaceutical compounds in environmental matrices occurs only in trace concentrations, adverse effects can occur on non-target organisms once these substances were created to be effective even at low concentrations and to be resistant to degradation[10].

Thus, human pharmaceuticals became to be identified and classified as emerging pollutants since it presents potential risk to aquatic ecosystems. Therefore, environmental directives European and North American has sought regulate the approval process, marketing and disposal of these substances[2,11].

In view of this, USEPA has launched the 'Water Quality Research Multi-Year Plan' in which was emphasized the need of research to support regulation and development of data for regulatory purposes concerning emerging contaminants (pharmaceuticals, endocrine disrupting substances, personal care products, and nanomaterials)[11]. So in the last decade, the effects of these emerging compounds on aquatic biota have been investigated through acute and chronic toxicity tests with different pelagic and benthic freshwater invertebrates, algae, and fishes[10,12,13].

Within this context, the aim of the present study was to evaluate the drugs: 17 α-ethinylestradiol, fluoxetine hydrochloride, diclofenac and ibuprofen using acute toxicity tests standardized with the cladoceran *Daphnia similis*. And thus, generate acute toxicity data to *D. similis* which can be used to contribute to the establishment of maximum environmentally safe concentration for these emerging compounds.

METHODS

Toxicity bioassays

The 17 α-ethinylestradiol (EE2) and the fluoxetine hydrochloride were exposed to test organisms *D. similis* in different concentrations (6.125; 12.5; 25; 50; 100% and 2.8, 3.4, 4.1, 5.0, 6.0, 7.1; 8.6 mg/L; respectively) and to negative control water. Simultaneously, were performed tests with controls solvent, DMSO to 17 α-ethinylestradiol and acetone for fluoxetine hydrochloride.

The diclofenac and ibuprofen were exposed to test organisms (*D. similis*) in different concentrations (10; 16; 25; 40; 65; 104 mg/L and 50; 80; 128; 205; 328; 500 mg/L, respectively) and to negative control of water. At the same time, were realized tests to negative control of Na_2CO_3 (ibuprofen diluents). Neonates were separated from stock cultures and placed individually at test tubes containing 10 mL of test medium. Four replicates were done for each treatment and negative controls. The test conditions followed the standardized method

NBR 12713/2004[14]. Acute bioassays were performed on a germination chamber set to 20 ± 1 °C, and the photoperiod was adjusted to 12h light/12h dark for the tests. After completion the assays were measured dissolved oxygen and pH.

Statistical analysis

The values to $EC50_{(48h)}$ (immobility) of 17 α-ethinylestradiol, fluoxetine, diclofenac and ibuprofen to *D. similis* was estimated by the *Trimmed Spearman-Karber* method[15], using nominal concentrations.

RESULTS AND DISCUSSION

In this study was observed that the survival of *D. similis* was affected by the treatment with the four pharmaceuticals tested. The means values for EC50 obtained to 17 α-ethinylestradiol and to fluoxetine were of 1.63 mg/L and 4.41 mg/L respectively. The means values for EC50 estimated for diclofenac and for ibuprofen were of 46.0 mg/L and 97.0 mg/L respectively.

Several studies have reported the potential risk that the pharmaceuticals offer to the aquatic organisms (Table 1).

Table 1. Mortality and immobility of Daphnia similis responses to treatments with: 17 α-ethinylestradiol, fluoxetine, diclofenac, ibuprofen, and their respective literature data

Compound	Taxon	Specie	Toxicological endpoint	Ecotoxicity data	Reference
17 α-Ethinylestradiol	Crustacean	*Daphnia similis*	$EC50_{(48h)}$	1630 µg/L	Present work
17 α-Ethinylestradiol	Crustacean	*Daphnia magna*	$NOEC_{(21d)}$ LOEC	500.0 µg/L 1.0 µg/L	16
17 α-Ethinylestradiol	Fish	*Fundulus heteroclitus*	$LOEC_{(14d)}$	0.05– 0.25µg/L	17
Fluoxetine	Crustacean	*Daphnia similis*	$EC50_{(48h)}$	4410 µg/L	Present work
Fluoxetine	Crustacean	*Daphnia magna*	$NOEC_{(21d)}$ LOEC	8.9 µg/L 31.0 µg/L	12
Fluoxetine	Mollusk	*Lampsilis siliquoidea*	$EC50_{(96h)}$	62.0 µg/L	18
Diclofenac	Crustacean	*Daphnia similis*	$EC50_{(48h)}$	46000 µg/L	Present work
Diclofenac	Crustacean	*Daphnia magna*	$EC50_{(48h)}$ $NOEC_{(21d)}$	67.0 µg/L 10.0 µg/L	19
Diclofenac	Fish	*Danio rerio*	NOEC	1131.0 µg/L	20
Ibuprofen	Crustacean	*Daphnia similis*	$EC50_{(48h)}$	97000 µg/L	Present work
Ibuprofen	Mollusk	*Phymorhynchus carinatus*	$LC50_{(72h)}$	17.1 µg/L	21
Ibuprofen	Fish	*Pelteobagrus fulvidraco*	$EC50_{(24h)}$	5.0 µg/L	22

Previous studies realized with 17 α-ethinylestradiol have reported the presence of this compound in several environmental matrices, such as in groundwater and in surface water, at range of 0.8 ng/L to 30.0 ng/L[8,23,24]. Although above the concentrations found in the environment the 17 α-ethinylestradiol proved to be capable of causing adverse effects to *D. similis*, as noted by the present study and for *D. magna*, as observed in the study realized by Clubbs[16].

Fluoxetine has been detected in environmental matrices in different countries, as

in sewage treatment system in Norway and in groundwater in USA, reaching limits of 0.4 ng/L to 56.0 ng/L[4,8].

The results presented in Table 1, show that the concentrations observed on toxicity tests in different test organisms exposed to fluoxetine were higher than the values observed in environmental matrices by Kolpin[8] in which the maximum concentration was 12.0 ng/L.

In terms of adverse effects of fluoxetine on reproduction of aquatic organisms, previous studies are contradictory: Fent[5] reported that an exposure at 0.036 mg/L of fluoxetine reduced significantly *D. magna's* fertility. In contrast, other studies reported an increase on reproduction rate of aquatic invertebrates exposed to fluoxetine[4]. On the present study were reported adverse effects to *D. similis* survival's when exposed to concentrations of 4.41 mg/L of fluoxetine hydrochloride.

For the diclofenac, a study in Brazil observed a concentration of 60.0 ng/L of this compound in river Paraiba do Sul which is a water catchment location to Rio de Janeiro city[2]. However in studies realized worldwide were identified maximum concentrations of diclofenac in environmental matrices above the values observed by Stumpf[2] in a range of 12.0 ng/L to 1.3 mg/L[6,23,25].

Although on study realized by Han[25] the chronic toxicity results indicate that the diclofenac was able to cause a decrease in rate of fecundity to *D. magna*, as well as observed in the present study in which the survival of *D. similis* was also affected by this compound, these effects were observed in concentrations above those found in environmental matrices.

To ibuprofen, concentrations found in environment were quantified in a range of 10.0 ng/L to 20.0 mg/L[2,6,8]. In the ibuprofen toxicity tests, performed with *D. similis* was reported average value for EC50 higher than the maximum values detected in the aquatic environment.

CONCLUSION

The acute toxicity tests with *Daphnia similis* showed an increased sensitivity to 17 α-ethinylestradiol, fluoxetine, ibuprofen and diclofenac respectively. Even presenting potential to cause adverse effects to aquatic biota, the results obtained to *D. similis*, presented means values to EC50 higher than the results reported by other studies for these same pharmaceuticals when found in environmental matrices. Thus, development of other studies still needed to assess potential toxic that these and others pharmaceuticals and their metabolites to might cause aquatic biota, with aiming to identify environmentally safe maximum concentrations that can guide future legislations.

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