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ELECTRON BEAM IRRADIATION OF COMBINED PHARMACEUTICALS: PROPRANONOL AND FLUOXETINE AND RELATED ECOTOXICITY

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ABSTRACT

There are serious evidences that justify the search for treatment technologies or processes combination for the improvement of decomposition for dozens of pharmaceuticals in wastewaters. Electron beam irradiation may play an important role in this scenario and relatively low doses have been reported for such purposes. The aim of the present study was to evaluate the toxic response of the crustacean *Daphnia similis* exposed to individual and combined pharmaceuticals. Several experimental trials of an acute immobilization test were performed with a mixture of pharmaceuticals composed of fluoxetine hydrochloride (Prozac®), and propranolol. Single pharmaceuticals were first tested separately. Toxicity of binary mixture was then assessed using five concentrations and 5 percentages of each substance in the mixture (0, 25, 50, 75, and 100%). Acute EC50% values ranged from 5.0 to 7.4 for fluoxetine and from 11.3 to 13.7 for propranolol. In mixture, values ranged from 6.4 to 9.8. Fluoxetine was more toxic for *D.similis* than propranolol. The different pharmaceuticals concentrations employed in a mixture showed no difference in toxicity values. When electron beam irradiation was applied, approximately 80% of acute effects were reduced at 5 kGy, and the mixture containing a higher percentage of fluoxetine, also showed a greater reduction of toxicity.

1. INTRODUCTION

Pharmaceuticals use is increasing worldwide, and they cause major impacts to the environment. As persistent contaminants, pharmaceuticals may contaminate water, soil and sediment. These emerging pollutants were found in studies with surface water, groundwater, soil and sediment at very low concentrations ng/L^{-1} to µg/L^{-1}, being able to cause changes in the endocrine system of aquatic organisms and damages to human health [1].

Pharmaceuticals enter the aquatic ecosystems for different via: human consumption, veterinary medicine, agricultural, and industrial routes, however, its main route of entry is through the sewage effluent [1]. When the pharmaceuticals are ingested, they can be excreted in a biologically active form, either as parent substance or as an active metabolite [2]. Mainly because of incomplete disposal at wastewater treatment plants, pharmaceutical residues and their metabolites occur in rivers, lakes and coastal waters and are also found in groundwater and drinking water [3,4]. They are continuously added and not efficiently removed, thus most of them exhibit pseudo-persistence [5].

The risk assessment of toxic substances in the aquatic environment focuses on the evaluation of a single substance, however, aquatic animals are exposed to mixtures of contaminants, the components of sewage may interact, producing synergistic, additive or antagonistic toxic effects [6]. Pharmaceuticals occur simultaneously in the aquatic environment, not as isolated contaminants [1, 3, 4]. Therefore, the joint effects of the mixtures must be considered and the risks to aquatic life have to deal with this complex exposure situation. The environmental consequences of pharmaceutical blends are identified as the primary need for research in order to understand the risks of long-term exposure to pharmaceuticals [7].

The propranolol is a beta-adrenergic blocker widely prescribed for the treatment of cardiovascular diseases, including hypertension, cardiac dysrhythmia and angina. In surface water, propranolol, bisoprolol and metroprolol were detected in concentrations of 0.59; 2.9 and 2.2 mg/L^{-1}, respectively [8] Environmental concentrations of propranolol reported are 1.9 $\mu g/L^{-1}$ in effluents and 0.59 $\mu g/L^{-1}$ in surface water, which may represent a risk for most sensitive freshwater species [9]. The propranolol presents high mobility in natural soils/sediments and capacity of accumulation in the aquatic ecosystems; Among beta-blockers, propranolol is the most hydrophobic and shows potential for bioaccumulation [10].

Fluoxetine is a selective serotonin reuptake inhibitor prescribed as an antidepressant worldwide for treating depression, obsessive-compulsive disorders, nervous bulimia, panic, and other psychological disorders [11]. A relatively high percentage of fluoxetine (approximately 20-30%) is excreted, unaltered by humans, and has been detected in aquatic environments [12]. 5.85mg/L^{-1} (influent) e 0.112mg/L^{-1} (effluent) [13]. Concentrations of fluoxetine ranging from 0.32 to 0.54$\mu g/L^{-1}$ in municipal effluent and surface water were reported [11]. Bioaccumulation of the compound has been demonstrated in fish and freshwater bivalves [14, 15, 16]. In crustaceans, antidepressants affect freshwater amphipod activity patterns and geohatic behavior, crayon aggression, and the reproduction and development of daphnids [11]. Were reported the ocular lateralization during the aggressive behavior of males *Betta splendins* exposed to Prozac® (fluoxetine); changes in fish feeding and reproduction behavior of *Pimephales promelas* [17,18].

Ecotoxicological tests can, therefore, be used as valuable tools for evaluating the toxicity of aqueous solutions containing pharmaceuticals, it is by-products, and other pharmaceutical compounds.

Advanced Oxidation Processes (POAs) have been reported as suitable alternative or complementary technology for wastewater treatment. Ionizing radiation for the abatement of pollutants may be obtained with electron beam accelerator (EBI) or gamma sources irradiators. This technology is based on oxidative and reducing molecules produced during water radiolysis and chemicals degradation as demonstrated by many authors.

The objective of this study was to assess the toxicity of pharmaceuticals and to apply Electron Beam Irradiation, in order to reduce the toxicity of pharmaceuticals in aqueous solution at a binary mixture of fluoxetine and propranolol.

2. MATERIALS AND METHODS

2.1. Reagents

Fluoxetine hydrochloride $C_{17}H_{18}F_3NO \cdot HCl$, 309.33g.mol^{-1}; -N-methyl-3-phenyl-3 - [(α, α, α-trifluoro-p-tolyl) oxy] propylamine hydrochloride; CAS number 56296-78-7.

Propranolol $C_{16}H_{21}NO_2$, 259.34g.mol^{-1}; (RS)-1-(isopropylamine)-3-(naphthalene-1-yloxy)-propan-2-ol; CAS number 318-98-9. Both pharmaceuticals are readily soluble in water, so no solvents were necessary for the preparation of stock and test solutions.

2.2. Irradiation procedure

A Dynamitron Electron Beam Accelerator was applied for the irradiations. The beam energy was fixed at 1.4 MeV during all the experiments. Liquid samples were irradiated using a batch system in borosilicate containers (Pyrex) a volume of 246mL was used in order to ensure a suitable beam penetration, 4mm thickness for aqueous samples. The recipients speed was 6.72m min^{-1} for samples passing under the electron beam. Absorbed doses were confirmed using a Perspex Harwell Red dosimeter, batch KZ-4034, with less than 5% variation.

2.3. Toxicity assays

The acute toxicity tests with *Daphnia similis* were performed according to Brazilian standard methods (NBR 12713/2009). The effect observed was the immobility to organisms after 48 hours of exposure to the samples. Both the toxicity test results were obtained based on the mean value of solutions concentration, which affects the exposed organism (EC50%), as well as the 95% confidence intervals, calculated using the statistical method "Trimmed Spearman Karber" [19].

3. RESULTS AND DISCUSSION

For the evaluation of biological effects, the toxicity of binary mixture of pharmaceuticals was used, with five concentrations and five percentages of each substance in the mixture (0, 25, 50, 75, and 100%). Clear concentration-response relationships were observed in all acute toxicity experimental trials. The average acute EC50% across the three trials was 11.9 for PRP, 5.9 for FLX. For the binary mixtures, three different combinations of pharmaceuticals concentration were exposed do dafnids, resulting as an average EC50% of 9.1%. The EC50 values obtained from each trial and their confidence intervals are reported in Table 1 and 2.

Table 1: EC50% of PRP and FLX for *D. similis* estimated for single pharmaceuticals by 48h acute immobilization tests.

	EC50%	
	Propranolol	**Fluoxetine**
First trial	11.3 (8.9-14.2)	5.0 (4.3-6.2)
Second trial	12.5 (10.6-14.9)	7.4 (6.2-8.7)
Third trial	13.7 (13.1-14.5)	6.8 (4.8-9.5)

Table 2: EC50% of three concentrations with a binary mixture of PRP and FLX for *D. similis* estimated by 48h acute immobilization tests.

	EC50%		
	75%PRP-25%FLX	**50%PRP-50%FLX**	**25%PRP-75%FLX**
First trial	9.4 (7.7-11.5)	9.8 (7.7-12.1)	8.5 (6.9-10.4)
Second trial	9.7 (7.4-11.3)	8.5 (6.8-10.6)	9.1 (7.4-11.3)

From Table 1 data, FLX may be considered more toxic than PRP. For the binary mixture, there was no difference in toxicity for the three different combinations of pharmaceuticals concentrations, with EC50% average of 9.1.

Previous studies showed that PRP and FLX exert acute effects only at levels that are several orders of magnitude higher than environmental concentrations, which range from ng/L to low µg/L [20]. In agreement, the acute tests performed in the present study with propranolol alone provided EC50 in the range from 9.0 to 10.9 mg/L^{-1}. Taking into consideration also the EC50 estimated in fluoxetine test, the values ranged from 1.0 to 1.4 mg/L^{-1}. Besides the fact that the number of pharmaceuticals found in the environment continuously increases, it is evident the importance of studying the effects of mixtures rather than of single pharmaceuticals.

In real effluents, pharmaceuticals are present in combination with dozens of compounds of similar use as well as with other contaminants. Pharmaceuticals have been designed to have specific effects on target organisms, and may interact with specific proteins or enzymes and the consequences of mixtures are not easily predictable. Furthermore mixtures are often reported and documented to behave differently from single compounds [20].

A recent report highlighted that FLX treatment was likely to increase PRP accumulation in digestive gland of mussels [16]. Moreover, propranolol not only binds to β-adrenergic receptors but also to 5-HT1 receptors in humans, acting as a serotonin (5-HT) antagonist [21].

After the ecotoxicological analysis of pharmaceuticals, the EBI process was applied in order to determine the toxicity removal efficiency of these compounds. 2.5 and 5 dose were used. Figure 1 reports the data of EC50%, in relation to the radiation dose applied for the five percentages of each substance in the mixture.

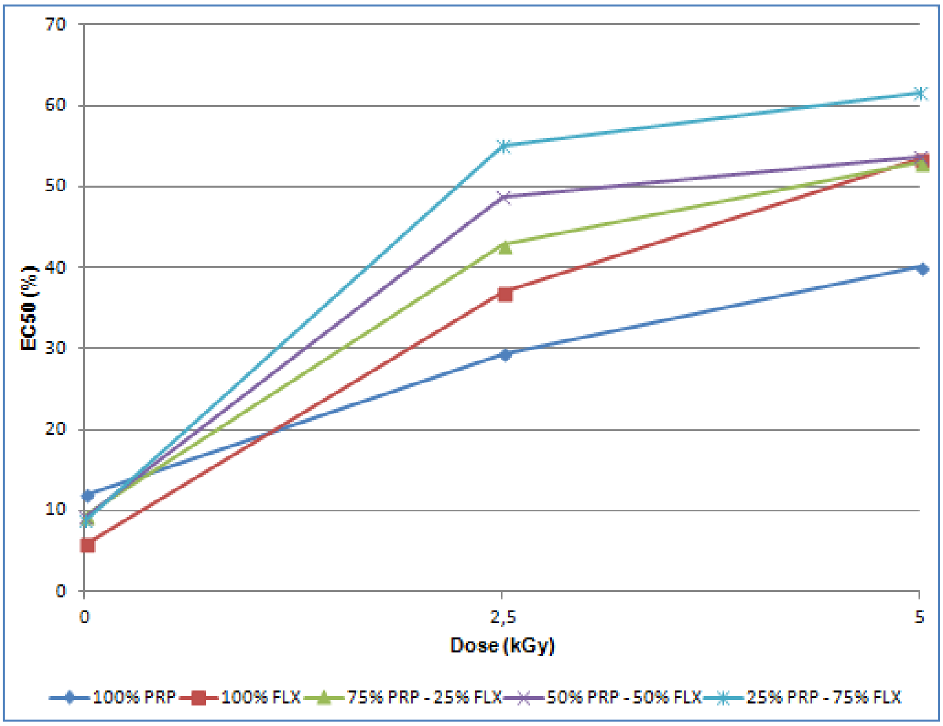

Figure 1: The EC50% of PRP and FLX in different concentrations versus dose.

EC50 values are inversely proportional to the effects, that means, the lower the value more toxic is the sample. There was a significant reduction of pharmaceuticals toxicity for both, isolated and in mixture.

When comparing the values of the isolated irradiated pharmaceuticals, FLX was more toxic to *D. similis* than PRP. Radiation efficiency for toxicity removal was higher for fluoxetine, isolated and mixtures of three different amount, Figures 1 and 2. Figure 2 shows the percentages to toxicity reduction after irradiation for three different concentrations pharmaceuticals in a mixture. It was noted a trend in the results, where the reduction of toxicity is slightly higher in the presence of larger amounts of fluoxetine.

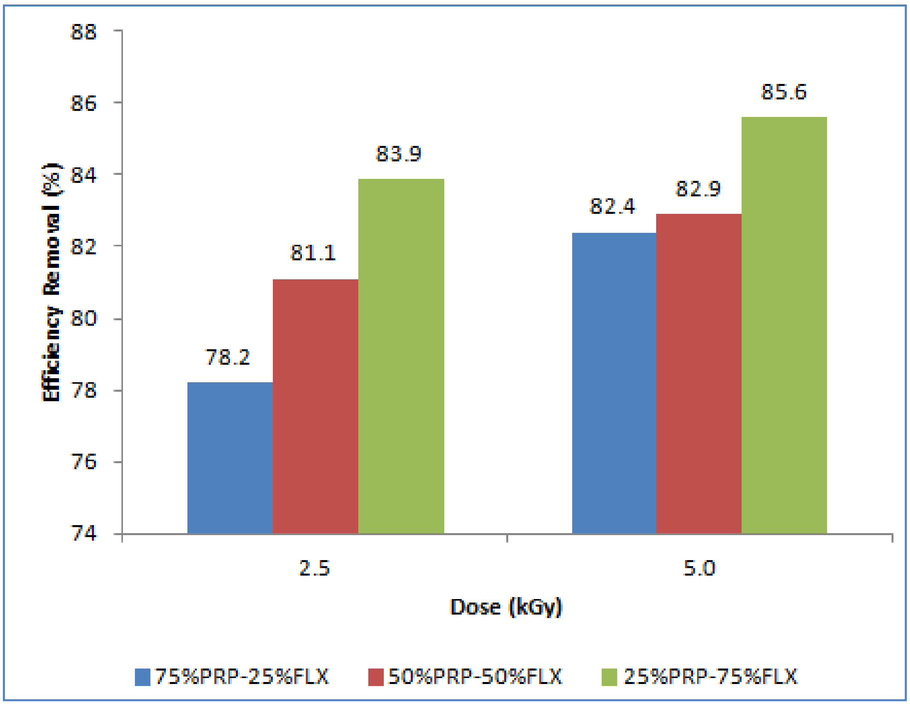

Figure 2: Efficiency Removal (%) of pharmaceuticals PRP and FLX in a mixture versus dose – 2.5 and 5 kGy.

When EBI was applied, approximately 80% of acute effects were reduced at 2.5 and 5 kGy.

From different authors who studied radiation effects into pharmaceutical solutions we noticed that at 5 kGy was a suitable dose for different authors: toxicity removal on mixture of fluoxetine hydrochloride and sodium dodecyl sulfate surfactant resulted in 91.89% for *Hyalella azteca;* 87.57% for *Daphnia similis* and 89.10%, for *Vibrio fischeri.* For samples of domestic sewage and its mixture with fluoxetine hydrochloride, 5 kGy reduced 100% and 79.32%, repectively. Mixture of pharmaceuticals diclofenac and fluoxetine hydrochloride the toxicity removal value was 66.9% at 5 kGy and exposed to *Daphnia similis* [22, 23, 24].

4. CONCLUSIONS

The pharmaceutical Fluoxetine hydrochloride was more toxic to the organism *Daphia similis,* than Propranolol. When electron beam treatment was applied the efficiency removal was 80% at the dose of 5 kGy. Three different pharmaceuticals concentrations were employed in a mixture, which showed no difference in toxicity values for *D. similis*. When applied EBI, was observed that the mixture containing a higher percentage of fluoxetine, also showed a greater reduction of toxicity – 83% and 85% in respectively doses of 2.5 and 5 kGy.

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