REDUCTION OF ACUTE TOXICITY OF THE PHARMACEUTICALFLUOXETINE (PROZAC®) SUBMITTED TO IONIZING RADIATION TO *Vibrio fischeri*

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ABSTRACT

The constant use of pharmaceutical drugs by great part of the population and its continuous input into the environment creates a growing need of investigating its presence, behavior and the effects on aquatic biota, as well as new ways to treat wastewater containing such substances. The fluoxetine hydrochloride (FH) present in the drug Prozac® is an active ingredient used in the treatment of depressive and anxiety disorders. Generally, these compounds enter the aquatic environment by sewage collectors systems after undergoing prior treatment in sewage treatment plants (STPs) or without any treatment. This study focused on evaluating the reduction of acute toxicity of the pharmaceutical FH, under its manipulated formula, for the marine bacterium *Vibrio ficheri*. It was also evaluated the acute toxicity of the aqueous solution containing the FH after its exposition to ionizing radiation from industrial electron accelerator. It was performed acute toxicity tests lasting 15 minutes, where the average EC (50) of the non-irradiated CF water solution was approximately 0.68 mg L^{-1}. While the CF water solution irradiated with 1 kGy, 2.5 kGy, 7.5 kGy and 10 kGy, presented an average EC(50) 1.63 $mg.L^{-1}$, 2.34 $mg.L^{-1}$, 2.35 $mg.L^{-1}$ and 1.80 $mg.L^{-1}$, respectively, showing a notable reduction of the acute toxicity for this organism.

1. INTRODUCTION

Pharmacology is the science responsible for studying the general pharmaceutical drugs, in all its aspects. Particularly it's responsible to describe, characterize, both the properties and preparation of several substances, besides of analyzing the action and effect generated by general drugs to different organisms. With the large-scale production and easy availability of the products generated by the pharmaceutical industry, the high consumption of pharmaceuticals and personal care products has become a widespread practice in urban society.

Due to enormous supply, uncontrolled use and constant disposal, there is an increasing number of wastewater containing pharmaceutical drugs and a significant increase of these "pollutants" in potable and surface water bodies [1].

Among the many chemicals developed trough years, one of them can be highlighted due to its broad commercialization, its effects to aquatic organisms and its presence in various environmental compartments. This pharmaceutical drug is the Benzenepropanamine, N-methyl-γ-[4-(trifluoromethyl) phenoxy]- hydrochloride, commonly known as fluoxetine

hydrochloride (FH), molecular formula $C_{17}H_{18}F_3NO$. Worldwide, it is also known by the trade name Prozac®, the first drug of its category.

In 1974, the FH was first described as a selective inhibitor of the reuptake of serotonin (5-hydroxytryptamine or 5-HT) in the synaptic cleft and later it was recognized by the U.S. F.D.A (United States Food and Drug Administration) as an appropriate method to the treatment of depression disorders. The FH allows the permanence of the activity of serotonin in other postsynaptic 5-HT receptors, facilitating the mood enhancement [2] [3].

It is known that many groups of chemicals when present in water, even in low concentrations, are potentially toxic to both the welfare of many aquatic and terrestrial ecosystems and to human health.

Pharmaceutical drugs reach the water bodies after being excreted by the human organism with urine and feces, depending on the physicochemical characteristics of the substance administered [4]. Besides that, the disposal of these compounds with household waste is also a potential source to environmental contamination.

Assuming the rising risks of the contamination of water bodies and soil, it is extremely important to generate new type of treatments to different liquid effluents containing pharmaceutical residues, aiming the degradation of these compounds before they reach the environment. Therefore the need to improve wastewater treatment to critical effluents, new oxidation processes are continually developed and two examples of this technology are photo-Fenton (FeSO4) and photocatalytic process (TiO2 + Solar Energy) [5].

Currently, a promising technology for wastewater treatment is the use of ionizing radiation during the pre-treatment of highly toxic liquid effluents in order to increase its biodegradability [6]. Since it is considered that very toxic effluents can affect the whole system of treatment of a STP. Ionizing radiation has been shown to be effective for the decomposition of organic substances and environmental pollutants in sewage sludge and wastewater [7] [5] and therefore may be appropriate for pre-treatment of effluents containing pharmaceutical drugs.

The Aquatic Ecotoxicology, as well as the different fields of Ecotoxicology, involves knowledge of several areas of science such as chemical analysis and statistics, combined with biological response that is obtained by essays with living organisms, which gives it a multi-disciplinarity and capability to evaluate and characterize the occurrence of possible biological effects in different environmental conditions [8].

Used as an important tool in ecotoxicological studies, the toxicity tests are used to generate legal requirements for disposal of effluents, to subsidize the establishment of values for the water quality criteria and to evaluate the efficiency of sewage treatment systems, among other applications [9]. Corroborating with information above, toxicity assays employing the bacterium *Vibrio fischeri* is an important and easy method to quickly evaluate acute toxicity of aqueous solutions.

2. OBJECTIVES

The focus of the present study was to evaluate the acute toxicity of fluoxetine hydrochloride to marine bacteria *Vibrio fischeri* and to assess the toxicity of this pharmaceutical drug, after

the aqueous solution containing it was exposed to ionizing radiation, from the industrial electron accelerator, establishing radiation doses and irradiation conditions.

3. MATERIALS AND METHODS

3.1. FLUOXETINE HYDROCHLORIDE

Each drug capsule contains 20 mg of fluoxetine hydrochloride in its formulation, besides some excipients (aerosil, sodium lauryl sulfate, micro-crystalline cellulose and maize starch). The FH solubility is 14 $mg.mL^{-1}$ in water [10]. Prior to the toxicity tests and the radiation of FH in aqueous solution, it was prepared a 10 mg L^{-1} stock solution, by dissolving the entire content of one capsule into two liters of milli-Q water. This solution was stirred during 48 hours, approximately, to ensure the complete solubilization of the active substance. A magnetic stirrer was used during the solubilization of FH.

3.2. IRRADIATION OF TEST SOLUTIONS

The irradiations were performed at the Radiation Technology Center (CTR), Instituto de Pesquisas Energéticas e Nucleares (IPEN), São Paulo, Brazil. The FH drug aqueous solution (10 $mg.L^{-1}$) was irradiated at the Dynamitron electron beam accelerator and the energy machine was fixed at 1.4 MeV during the expriments. The 246 mL samples were taken to irradiation field contained in a glass recipient (Pyrex®), covered with plastic wrap. The applied radiation doses were: 1.0 kGy, 2.5 kGy, 7.5 kGy and 10 kGy.

3.3. *Vibrio fischeri* TOXICITY ASSAYS

The acute toxicity tests with *Vibrio fischeri* were performed following the recommendations of the normative [11]. First, it was performed the reactivation of the bacteria, which are purchased in the lyophilized form. The entire content of a vial containing the bacteria was passed to a glass cuvette and 1000 mL of buffer reactivation solution was added.

After the bacteria be reactivated, another dilution of the reactive reagent (bacteria) was performed using diluent solution (1:10) Biolux solution. The sample preparation, before initiating the assay included the control group, which is the pure dilution solution (1000 mL) in a cuvette. The sample concentrations used for the acute toxicity tests were equivalent to 81.9%, 40.95%, 20.47% and 10.23%, following a serial factor 2 dilution. Two sequences of cuvettes were prepared in the luminescence analyzer: the first of them contained the samples and the suitable osmotic adjustment. The second line of cuvettes received the bacteria and the first signs of luminescence were measured in order to calibrate the analyser. The I_0 counting were taken from this second line cuvettes. After that each cuvette received the correspondent sample in order to get the I_{15}, after the exposition.

3.4. STATISTICAL ANALYSIS

The statistical analysis for *Vibrio fischeri* acute toxicity tests were based on the gamma value (ratio between the light lost and the remanescent light from each pair) and the concentration of the sample, with the 7.82 version of the program developed by Microbics Corp®. The program traces a curve that determines the Effective Concentration that reduced 50% of the light (EC50), the concentration of the sample which correspond to gamma value equal to 1, using linear regression [12].

4. RESULTS AND DISCUSSION

The results obtained on acute toxicity tests for the FH are shown in Table 1, while the results of the irradiated samples are presented in Table 2.

Table 1. Acute toxicity of the original FH to *Vibrio fischeri* (EC50, mg. L^{-1}, 15 min)

Essay	EC50 (mg.L^{-1})	Confidence interval (mg.L^{-1})
1	0.75	0.18 – 2.98
2	0.91	0.37 – 2.20
3	0.39	0.15 – 0.97

Table 2. Acute toxicity of the irradiated FH to *Vibrio fischeri* (EC50, mg. L^{-1}, 15 min)

Essay	Dose (kGy)	EC50 (mg.L^{-1})	Confidence interval (mg.L^{-1})
1	1	1.69	0.71 – 4.01
2	1	1.57	0.45 – 5.57
1	2.5	2.56	1.08 – 6.02
2	2.5	2.12	0.66 – 6.80
1	7.5	3.07	0.27 – 33.90
2	7.5	1.62	0.46 – 5.68
1	10	1.71	0.55 – 5.28
2	10	1.90	0.69 – 5.24

The reduction of bioluminescence emitted by *Vibrio fischeri* was the basis for this study.

Pharmaceutical drugs are active and persistent substances, these characteristics give them the ability to remain unchanged in different environmental matrices for years, constituting a potential risk to ecosystems, including public health. Understandings of the possible effects of drugs to different living organisms are increasing as well as new techniques for liquid effluents treatment are being developed.

At Table 3 there is a comparison between FH presences upstream and downstream from STPs. The low removal efficiency for different antidepressants may be highlighted.

Table 3. Environmental concentrations ($ng.L^{-1}$) of psychotropic drugs and their metabolites in sewage treatment plants downstream of major rivers in the metropolitan area of Madrid [13] (modified).

	Jarama	Manzanares	Guadarrama	Henares	Tajo	Median Values
Citalopram	3 -32	58	13 – 120	<D.L.	<D.L.	43
Fluoxetine	13 - 18	22	8 – 44	11	12	14
Venlafaxine	43-225	40 - 387	46 – 347	43	22	57

<D.L.: below detection limit

The occurrence of pharmaceutical residues in sewage and natural waters is an important topic discussed all around the globe. Studies show that these pharmaceutical drugs and their metabolites are present in aquatic environments in various parts of the world, including Germany, Brazil, Canada, Holland, Italy, Sweden, USA and UK.

Furthermore, investigations on the contamination of different aquatic environments by pharmaceutical drugs show that these residual contaminants are present in concentrations of $\mu g.L^{-1}$ and $ng.L^{-1}$ [4].

Among the different classes of pharmaceuticals that have been reported it can be highlighted the antibiotics [14], natural and synthetic estrogens [15] and antidepressants [13].

The use of aquatic species in toxicity assays for the evaluation of acute effect is closely related to the fact that coastal regions or regions that are located on the banks of rivers receive large amount of contaminants from industrial effluent and domestic sewage.

Comparing the results of acute toxicity for the FH irradiated with different radiation doses to the non-irradiated FH, some important reduction may be observed (Figure 1).

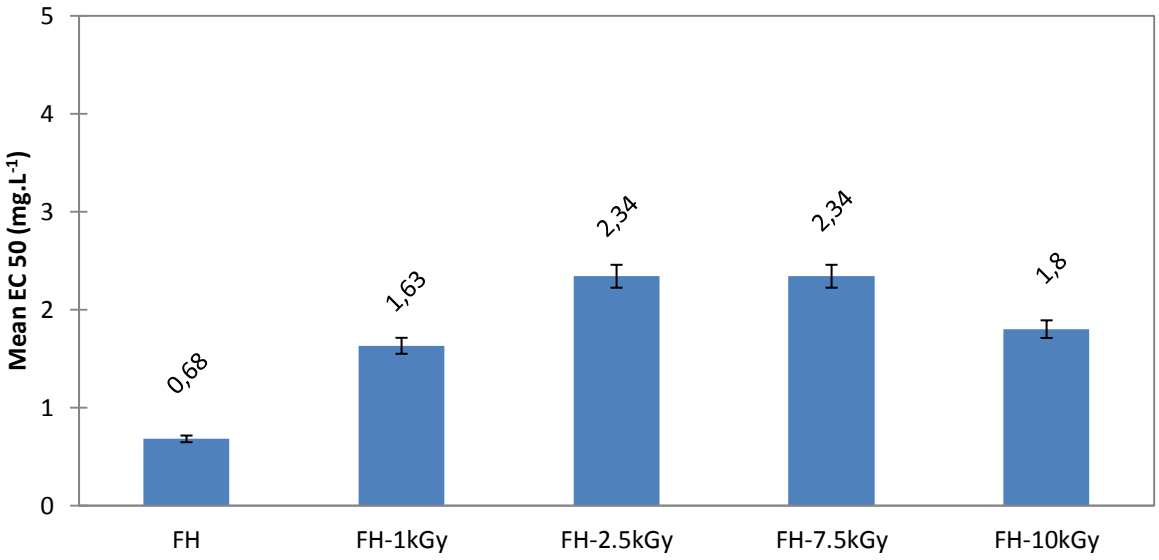

Figure 1. Average EC50 values obtained by acute toxicity assays to *Vibrio fischeri*.

The best result for the acute toxicity was obtained at 2.5 kGy (irradiated FH was 2 times less toxic).

These results can be explained by a possible breakdown or transformation of the fluoxetine hydrochloride molecule, mainly by hydroxyl radicals formed by radiolysis of water and in part by the direct irradiation of the molecule. The indirect photolysis of FH by oxidation with hydroxyl radicals in simulated natural water has shown to be faster than by direct photolysis [16].

Acute toxicity tests with the crustacean *Daphnia similis*, the bacteria *Vibrio fischeri* and the fish *Poecilia reticulata* were used to evaluate the toxicity of industrial and domestic effluents exposed to ionizing radiation and it was obtained a significant reduction of the toxicity [7]. The ionizing irradiation was used as well to reduce the toxicity of the surfactants linear alkylbenzene sulfonate (LAS) and sodium dodecyl sulfate (DSS) to aquatic living organisms being obtained a significant reduction of chronic toxicity to *Ceriodaphnia dubia* and acute toxicity to *Vibrio fischeri* with radiation doses 3.0 kGy and 6.0 kGy, which are considered to be relatively low doses [5].

4.1. FLUOXETINE HYDROCHLORIDE AND REGULATIONS

Fluoxetine hydrochloride has been marketed for over 35 years, and its prescription and use has increased over time. In addition, several similar pharmaceutical drugs of generic formulation have also been commercialized from past few years to nowadays.

To ensure a high level of protection to human and to environmental health, the European Union began implementing the Registration, Evaluation, Authorization and Restriction of Chemical substances(REACH), a single integrated system created by the European Chemicals Agency which requires companies that manufacture (when the volume produced

exceeds the value of one tone per year) and import chemicals to assess the risks arising from the use thereof and to take necessary measures to manage any risks they identify [17].

Allied to this policy is the European directive 93/67/EEC of 1993, which classifies substances according to the results of toxicity (EC50, IC50) to aquatic organisms. This directive classifies different chemicals as "extremely toxic" when EC50 < 0.1 $mg.L^{-1}$, "very toxic" if $0.1 \leq EC50 \leq 1$ $mg.L^{-1}$, "toxic" when $1 < EC50 \leq 10$ $mg.L^{-1}$, "dangerous" if $10 < EC50 \leq 100$ $mg.L^{-1}$ and "non toxic" when EC50 values obtained are above 100 $mg.L^{-1}$ [18] [19]. Thus, in Table 4 is presented the classification of the fluoxetine hydrochloride, when taken as reference the results obtained in this study and the European Directive 93/67/EEC, 1993.

Table 4. Classification of the FH, based on the acute toxicity results obtained to *Vibrio fischeri*, according to EU Directive 93/67/EEC, 1993.

	Extremely Toxic	Very Toxic	Toxic	Dangerous	Non toxic
FH		X			
FH - 1 kGy			X		
FH - 2.5 kGy			X		
FH - 7.5 kGy			X		
FH - 10 kGy			X		

5. CONCLUSIONS

This study shows that acute toxicity effects caused by the FH to *Vibrio fischeri* were observed at an average concentration 0.68 $mg.L^{-1}$. It was also observed that there was a reduction of the FH toxicity (2 times less toxic) to *Vibrio fischeri*, after the drug water solution was exposed to ionizing radiation dose 2.5 kGy.

Nevertheless, it is important to state that even after this reduction of the FH acute toxicity to *Vibrio fischeri*, it still can be classified as a toxic compound if present in water bodies according to the EU Directive 93/67/EEC.

Thus, it's important to encourage further studies that aim the minimization of the impact caused by pharmaceutical residues to human and environmental health.

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