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RESEARCH ARTICLE

Degradation and acute toxicity removal of the antidepressant Fluoxetine (Prozac®) in aqueous systems by electron beam irradiation

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Abstract Electron beam irradiation (EBI) has been considered an advanced technology for the treatment of water and wastewater, whereas very few previous investigations reported its use for removing pharmaceutical pollutants. In this study, the degradation of fluoxetine (FLX), an antidepressant marketed as Prozac®, was investigated by using EBI at FLX initial concentration of 19.4 ± 0.2 mg L^{-1}. More than 90 % FLX degradation was achieved at 0.5 kGy, with FLX below the detection limit (0.012 mg L^{-1}) at doses higher than 2.5 kGy. The elucidation of organic byproducts performed using direct injection mass spectrometry, along with the results of ion chromatography, indicated hydroxylation of FLX molecules with release of fluoride and nitrate anions. Nevertheless, about 80 % of the total organic carbon concentration remained even for 7.5 kGy or higher doses. The decreases in acute toxicity achieved 86.8 and 9.6 % for *Daphnia similis* and *Vibrio fischeri* after EBI exposure at 5 kGy, respectively. These results suggest that EBI could be an alternative to eliminate FLX and to decrease residual toxicity from wastewater generated in pharmaceutical formulation facilities, although further investigation is needed for correlating the FLX degradation mechanism with the toxicity results.

Keywords Acute toxicity · Degradation · Electron beam irradiation · Fluoxetine · Radiolysis

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Highlights
- The feasibility of electron beam irradiation (EBI) to remove fluoxetine (FLX) from water is shown for the first time.
- The effect of the radiation dose needed to remove FLX and acute toxicity was investigated.
- EBI resulted in the reduction of acute toxicity of FLX solutions at low irradiation doses.
- Byproducts were elucidated and the pathway of FLX degradation by EBI is proposed.

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Introduction

The presence of pharmaceutical compounds in aquatic environments even at relatively low concentrations (ng L^{-1} to μg L^{-1}) represents an emerging environmental issue. Many studies have shown that most of these contaminants are not completely degraded in sewage and wastewater treatment plants (WWTPs) and then may reach surface water (Segura et al. 2013). Discharges of untreated residues from livestock and land application of biosolids from WWTPs are also considered contamination sources (Wu et al. 2010). Moreover, concentrations up to mg L^{-1} have been reported for effluents from pharmaceutical formulation facilities and drug manufactures (Larsson et al. 2007; Lester et al. 2013).

The variety of pharmaceutical pollutants includes fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI) known as Prozac®, used for treating depression and obsessive-compulsive disorders. This drug has been detected in surface waters in the USA and Canada at 0.012 and 0.013–0.046 μg L^{-1}, respectively (Kolpin et al. 2002; Metcalfe et al. 2003); in sewage treatment plant effluents at 0.038–

0.099 μg L^{-1} in Canada (Metcalfe et al. 2003; Hua et al. 2006), 0.0017 μg L^{-1} in South Korea (Kim et al. 2007), and 0.0006–0.0187 μg L^{-1} in sewage in Norway (Vasskog et al. 2008); in wastewater at 0.021 μg L^{-1} (USA) (Glassmeyer et al. 2005) and wastewater treatment plants effluents at 0.020–0.091 μg L^{-1} (Canada) (Metcalfe et al. 2010); in biosolids and sediments (USA) at 37.4 and 1.84 μg kg^{-1}, respectively (Furlong et al. 2004), and in solids obtained after clarification (49.5 μg kg^{-1}) and sand/GAC filtration (58.6 μg kg^{-1}) processes in a drinking water treatment plant in the USA (Stackelberg et al. 2007). FLX has also been found in drinking water (<0.014 μg L^{-1}) (USA) (Stackelberg et al. 2007). Based on a database derived from French and international journals, Deblonde et al. (2011) reported average influent and effluent FLX concentrations in WWTPs of 5.85 and 0.11 μg L^{-1}, respectively; however, the corresponding removal (98.1 %) is most likely due to adsorption onto biosolids (Méndez-Arriaga et al. 2011; Lajeunesse et al. 2012). In fact, FLX is not expected to biodegrade rapidly as shown by different authors (Kwon and Armbrust 2006; Redshaw et al. 2008; Méndez-Arriaga et al. 2011).

FLX has a half-life of about 7 days in pure water exposed to natural sunlight (Lam et al. 2005) and has been reported as toxic at low concentrations to several aquatic organisms and also as a potential endocrine disruptor (Brooks et al. 2003). The drug has been found in muscle tissues, liver, and brain of fishes collected downstream of effluent discharges (Brooks et al. 2005) and can induce spawning in some crustaceans and bivalves species (Fong 1998). Long-term persistence and bioaccumulation of FLX were reported for *Oryzias latipes* fishes (Nakamura et al. 2008) and bull sharks (*Carcharhinus leucas*) (Gelsleichter and Szabo 2013). Gaworecki and Klaine (2008) evidenced that the ability of the hybrid fish species striped bass (*Morone saxatilis* × *Morone chrysops*) to capture prey decreased after a 6-day exposure to FLX (23.2-100.9 μg L^{-1}). Another important issue is the ability of FLX to readily react with hypochlorite in pure water and in wastewater, forming the active chloramine *N*-chlorofluoxetine, which shows increased hydrophobicity and tendency to adsorb on sediments, soils, and biological membranes (Bedner and MacCrehan 2006). These and other evidences contributed to the recent inclusion of FLX in a list of 10 pharmaceuticals potentially dangerous for the environment (Santos et al. 2013).

Limitations of primary and secondary processes in WWTPs to eliminate pharmaceuticals have boosted the development of alternative treatment strategies. Li et al. (2012) studied the degradation of different pharmaceuticals and personal care products (PPCPs) by the Fenton process and found that FLX (at an initial concentration of 1 μg L^{-1}) was completely degraded within 30 minutes with H_2O_2/Fe (II) = 0.5 (mol/mol). Méndez-Arriaga et al. (2011) studied FLX degradation by UV-irradiated and non-irradiated oxidation processes (TiO_2, O_3, $O_3 + H_2O_2$, $TiO_2 + O_3$, and $TiO_2 + O_3 + H_2O_2$). The authors found that the TiO_2/UV process resulted in complete depletion of FLX (initially at 34 mg L^{-1}) and 50 % mineralization within 60 min; the latter could be increased to more than 70 % by adding H_2O_2. Ozonation alone resulted in FLX removal within 10 min, while 97 % mineralization was achieved within 60 min by the $O_3 + H_2O_2$/UV process. By contrast, Uslu et al. (2012) obtained only 58 % FLX removal from Lake Huron water using the O_3/H_2O_2 treatment. Finally, Serna-Galvis et al. (2015) investigated FLX degradation by sonochemical degradation coupled to biological treatment. The authors showed that FLX could not be removed by microorganisms, even after 5 days under favorable conditions of pH (7.0) and temperature (37 °C). The sonochemical treatment (600 kHz, 60 W) readily eliminated FLX, with only 15 % mineralization after 360 min of sonication. After this previous treatment, 70 % of the initial TOC was removed in the biological system.

These processes have found, however, limited full-scale application owing to the need of addition of different chemicals, strict pH ranges, and sludge generation (in the case of Fenton and photo-Fenton reactions) and mainly to the difficulty in treating water and wastewater at high flow rates (Parsons 2004).

Among alternative advanced treatment techniques, electron beam irradiation (EBI) is based on water radiolysis, a process able to generate hydroxyl radicals (HO·), hydrogen atoms (H·), electronically excited species, ionized molecules, and aqueous electrons (e^-_{aq}). These species promote oxidation, reduction, dissociation, or degradation of organic pollutants (Cooper et al. 2004). The efficacy of electron beam accelerators for degrading pollutants of various chemical natures, e.g., 4-chlorophenol (Yang et al. 2007), atrazine (Xu et al. 2015), and textile dyes (Han et al. 2012), as well as for removing residual toxicity from water containing surfactants (Romanelli et al. 2004) and from effluents of municipal and industrial wastewater treatment plants (Borrely et al. 2004) has been evidenced. Han et al. (2012) shortly describe an industrial plant with an electron beam accelerator of 1 MeV and 400 kW, for treating 10,000 m^3 of textile dyeing wastewater per day (Daegu Dyeing Industrial Complex, South Korea). According to the authors, the EBI treatment at around 1 kGy improves the biodegradability of the treated effluent, therefore decreasing the retention time in the combined biological treatment process; the treatment cost of the EBI system was about USD 0.3 per cubic meter of wastewater.

In the case of pharmaceuticals, Homlok et al. (2011) demonstrated the elimination of diclofenac from water, at initial concentrations of 0.1–1.0 mmol L^{-1} using ^{60}Co gamma-irradiation at a dose of 1 kGy; partial mineralization of transformation byproducts and expressive decrease in toxicity were achieved at 5–10 times higher doses. Based on the electric energy in kWh required to degrade a contaminant by one order of magnitude in a unit volume (EE/O), Kim et al. (2012) found

EBI to be energetically effective for the removal of sulfamethoxazole and chlortetracycline in comparison with ozone and UV (254 nm)-based processes, with 88.6 and 100 % antibiotic removals, respectively, at 1 kGy. The toxicity indicator used by the authors (50 % cell growth inhibition concentration for the green algae *Pseudokirchneriella subcapitata*) for both antibiotics increased with increasing dose in the range 0–5 kGy. Despite these positive results, the use of electron beam irradiation technologies for treating water and wastewater containing pharmaceutical compounds has not yet been extensively studied.

In this study, we investigated the use of an electron beam accelerator for degrading FLX (Prozac®) in aqueous solution. To our knowledge, this is the first study to report experimental results on the use of EBI for this purpose, exploring the radiation dose needed to remove the target pollutant and to decrease acute toxicity. Moreover, the elucidation of transformation byproducts is discussed and the pathway of FLX degradation by EBI is proposed.

Experimental

Chemicals

Fluoxetine hydrochloride [$C_{17}H_{18}F_3NO{\cdot}HCl$, methyl[(3S)-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propyl]amine] (CAS 54910-89-3) (Divis Pharmaceuticals Pvt. Ltd., 98.8 %) was used as a standard in chromatographic analysis and in all the experiments. Acetonitrile and acetic acid (HPLC grade) were purchased from Sigma-Aldrich. All the solutions used in EBI experiments were prepared using ultra-pure water (Millipore Milli-Q).

Irradiation procedure

In all experiments, electron beam irradiation (EBI) was performed using a Dynamitron® Electron Beam Accelerator at 37.5 kW and 1.4 MeV. Radiation doses ranged from 0.5 to 7.5 kGy; some irradiations were also performed at 20 kGy. Doses were measured using a Perspex Harwell Red, Batch KZ-4034 dosimeter, with less than 5 % variation. Aqueous solutions (246 mL) containing FLX ($[FLX]_0 = 19.4 \pm 0.2$ mg L^{-1}) were placed in rectangular glass recipients and irradiated in batch, with a maximum exposed liquid depth of about 4 mm; these recipients were submitted to the electron beam at 6.72 m min^{-1}. An additional EBI experiment was performed using a FLX aqueous solution at the same initial concentration, which was diluted in raw domestic sewage (50 % *v*/*v*). All the experiments were performed at room temperature and initial pH 6, at which the FLX molecule is in its protonated form ($FLX-H^+$) (pKa = 10.1, Bedner and MacCrehan 2006). Diluted NaOH or HCl solutions were used to adjust pH to the initial value; pH was not corrected over reaction time. Standard deviations were calculated from two replicates of each experiment.

Analytical methods

Ultra-fast liquid chromatography

FLX concentrations were determined by ultra-fast liquid chromatography (UFLC) using a Shimadzu equipment (LC 20AD), with a fluorescence detector (RF-10Axl) and a C_{18} column (Kinetex Phenomenex, 150 mm × 4.6 mm, 5 μm). The oven temperature was 40 °C. The eluents were (A) acetic acid 1 % and (B) acetonitrile at 70:30 ratio and 1.00 mL min^{-1} flow rate; isocratic analysis was used. For fluorescence analysis, the wavelengths for excitation and emission of FLX were 230 and 290 nm, respectively. In these conditions, the retention time of FLX was 5 min. Calibration was carried out using external standards prepared with known concentrations of FLX. Two calibration curves were used depending on the concentration range: curve 1 ($R^2 = 0.999$; DL = 0.53 mg L^{-1}; QL = 1.61 mg L^{-1}) and curve 2 ($R^2 = 0.998$; DL = 0.012 mg L^{-1}; QL = 0.035 mg L^{-1}), where DL and QL refer to detection and quantification limits, respectively. The corresponding sample injection volumes were 7.0 and 50 μL, respectively.

Total organic carbon

Total organic carbon (TOC) of selected samples was measured using the Shimadzu TOC-5000A equipment.

Ion chromatography

Concentrations of inorganic ions (F^-, NO_3^-, NH_4^+) were determined at room temperature by ion chromatography (IC) using a Metrohm equipment (model 851) with an electrical conductivity detector. Analytical conditions for anion determination were as follows: Metrosep A-Supp 5-Metrohm anion column (250 × 4 mm), eluent solution (4.0 mmol L^{-1} Na_2CO_3/1.0 mmol L^{-1} $NaHCO_3$), flow of 0.7 mL min^{-1}, suppressor column (Metrohm), and regenerative solution (50 mmol L^{-1} H_2SO_4). For cations, the analytical conditions were: Metrosep C2-150-Metrohm cation column (150 × 4 mm), eluent solution (4 mmol L^{-1} tartaric acid/0.75 mmol L^{-1} dipicolinic acid), flow of 1.0 mL min^{-1}, and an electronic suppression system (Metrohm). Analytical quantification was performed using an external calibration curve obtained with standard solutions. The detection limits were below 1.0 μmol L^{-1} for F^-, NO_3^-, and NH_4^+ ions.

Toxicity assays

Acute toxicity assays were performed with the crustacean *Daphnia similis* and luminescent bacteria *Vibrio fischeri*. In both cases, the assays were carried in triplicate according to the ABNT Brazilian standards (ABNT 2009, 2012). Immobility of *D. similis* after 48 was the end point measured for this assay (ABNT 2009). Daphnids were cultivated in laboratory, and the organisms were exposed to several dilutions of irradiated and non-irradiated FLX solutions for 48 h. *V. fischeri* bioluminescence was measured using a Microbics 500® photometer, and four sample dilutions were measured after a 15-min exposure time. FLX concentrations of non-irradiated solutions used in assays performed with *D. similis* and *V. fischeri* were 10 and 5 mg L^{-1}, respectively; in both cases, the pH of irradiated solutions was previously corrected to 7.0. The EC50 values were calculated using standard statistical procedures; standard deviations were calculated from three replicates of the measurements carried out for each toxicity assay.

Identification of degradation products

Organic byproducts generated from FLX degradation by EBI at 1 kGy were identified by direct injection mass spectrometry (UHR-QqTOF, Ultra-High Resolution Qq-Time-of-Flight, impact II, Bruker Daltonics). The time-of-flight mass spectra were obtained in positive electrospray (+ESI) mode in the range *m/z* 50–1300 at the following optimized operating conditions: nebulizer 3.0 bar, dry gas 8.0 L min^{-1}, dry heater 220 °C, and capillary 4500 V.

Results and discussion

Fluoxetine degradation by EBI

Control experiments in the dark, at room temperature, showed negligible fluoxetine (FLX) hydrolysis at pH 6 (data not shown), in line with previous studies (Lam et al. 2005; Méndez-Arriaga et al. 2011). The evolution of FLX concentration, TOC, pH, and solution conductivity with dose are shown in Fig. 1. More than 90 % FLX degradation was achieved at only 0.5 kGy, whilst FLX concentrations below the detection limit (DL = 0.012 mg L^{-1}, analytical curve 2) were obtained at 2.5 kGy or higher. The reaction of FLX molecules with HO·, H·, or e^-_{aq} is a bimolecular process, with the overall rate depending on the contaminant and radical concentrations. The product of the radical species concentration and the respective second-order rate constant corresponds to a pseudo first-order rate constant (k_0), which can be expressed with respect to absorbed radiation dose rather than time (Cooper et al. 2004).

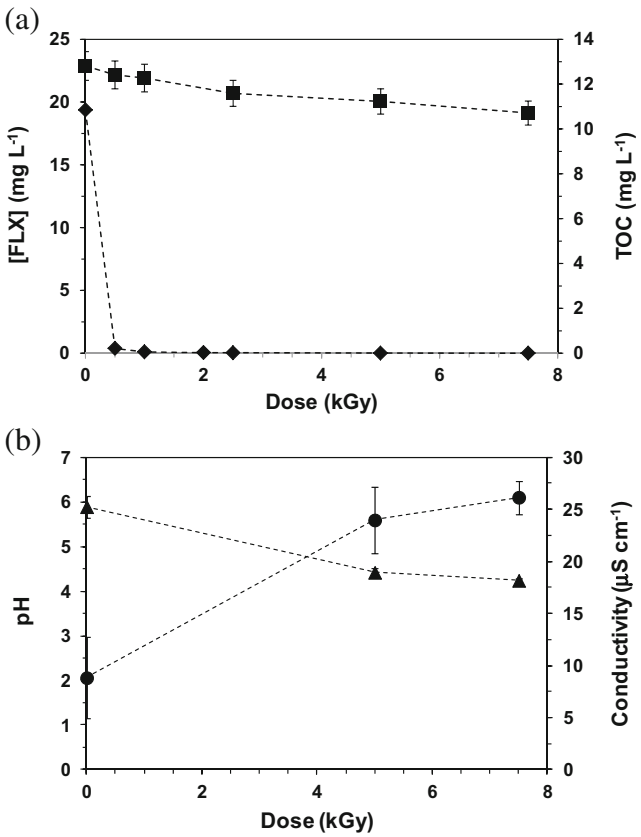

Fig. 1 (**a**) Fluoxetine and TOC concentrations vs. dose using electron beam irradiation (EBI): (*diamond*) [FLX]; (*square*) TOC. (**b**) Solution pH and conductivity vs. dose: (*triangle*) pH; (*circle*) conductivity. Initial FLX concentration: $[FLX]_0 = 19.4 \pm 0.2$ mg L^{-1}; initial TOC concentration: $TOC_0 = 12.8 \pm 0.1$ mg C L^{-1}; initial pH: 6

For doses lower than 1 kGy, a value of $k_0 = 5.74$ kGy^{-1} can be obtained using the first three data points in a graph of $\ln([FLX]/[FLX]_0)$ vs. dose, with $R^2 = 0.910$. From this k_0 value, the doses required to achieve 50, 90, and 99 % FLX removals from water can be estimated as 0.12, 0.40, and 0.80 kGy, respectively, which is important information in the application of EBI-driven treatment processes (Cooper et al. 2004).

These results can be compared with those obtained by Westerhoff et al. (2005) and Snyder et al. (2006), who also reported FLX removals higher than 90 % during ozonation and advanced oxidation. The substantially lower removal (58 %) obtained by Uslu et al. (2012) during O_3/H_2O_2 treatment of contaminated lake water (pH ~8) can be attributed to the different conditions at which the authors performed their experimental runs (e.g., different dosages and/or reaction times). Furthermore, according to Uslu et al. (2012), adsorption of positively charged FLX molecules onto negatively charged colloidal particles might limit the effectiveness of oxidation processes in real water and wastewater matrices.

Figure 1b shows a decrease in the solution pH from 6 to about 4.2 at 7.5 kGy, which is associated with the

formation of acidic transformation products; the pH after irradiating the solution at 20 kGy was about 3.8. These trends are also associated with the increase in the oxygen-to-carbon ratio in the degradation products, as well as the conversion of carbon bound fluorine and nitrogen to inorganic ions (Homlok et al. 2011). Opening of the hydroxylated aromatic ring formed upon the addition of the HO· radical and formation of low molecular weight organic compounds, including organic acidic species, are common steps during degradation of contaminants containing aromatic groups (Guo et al. 2012; Batista et al. 2014).

The decrease in pH with increasing dose has been previously reported for treating dye-contaminated wastewater exposed to electron beam (Vahdat et al. 2010). Furthermore, as detailed later, Lam and co-workers (2005) reported that under Xe lamp radiation FLX photolyzed to O-dealkylated and potentially to carboxylic acid photoproducts. As discussed by Garrido et al. (2009)) and Méndez-Arriaga et al. (2011), oxidation at the secondary amine and substituted aromatic ring forms unstable cation radicals, leading to FLX dimers; hydroxylation of the phenyl rings constitute the main pathway to aliphatic acids and inorganic species.

Figure 1b also shows that solution conductivity increased from 8.8 to 26.2 μS cm^{-1} with increasing dose (29.2 μS cm^{-1} at 20 kGy), in close relationship with the increased concentration of dissolved ions. In fact, Fig. 2a shows a significant increase in the concentration of fluoride anions in the solution with doses up to 2.5 kGy, at which [F^-] achieved 59.7 μmol L^{-1}, and then increased slowly to about 66 μmol L^{-1} at 7.5 kGy. This value indicates that about 30 % of the expected stoichiometric amount of fluorine initially present in FLX molecules was released to the solution as fluoride anions, even after the complete degradation of the target antidepressant ([FLX] below the detection limit of the chromatographic method, i.e., 0.012 mg L^{-1}). In other words, the results suggest the existence of recalcitrant fluorinated organic intermediates in the solution despite the dose. These findings are in agreement with Méndez-Arriaga et al. (2011), who identified the formation of defluorinated quinonoid-type species associated with the detachment of 1/3 of the fluorine atoms from FLX molecules through different oxidation processes (TiO_2-mediated photocatalysis, ozonation, and O_3/H_2O_2). In the case of ozonation and O_3/H_2O_2 processes, the authors observed that the formation of F^- ions increased at first and subsequently decreased, which was associated to re-fluorination of intermediate species at longer treatment times. Different reaction mechanisms were proposed for direct and indirect photolysis (Lam et al. 2005; Méndez-Arriaga et al. 2011). In our study, irradiations performed at higher doses up to 20 kGy indicated that F^- concentration did not show any decrease with increasing dose (results not shown).

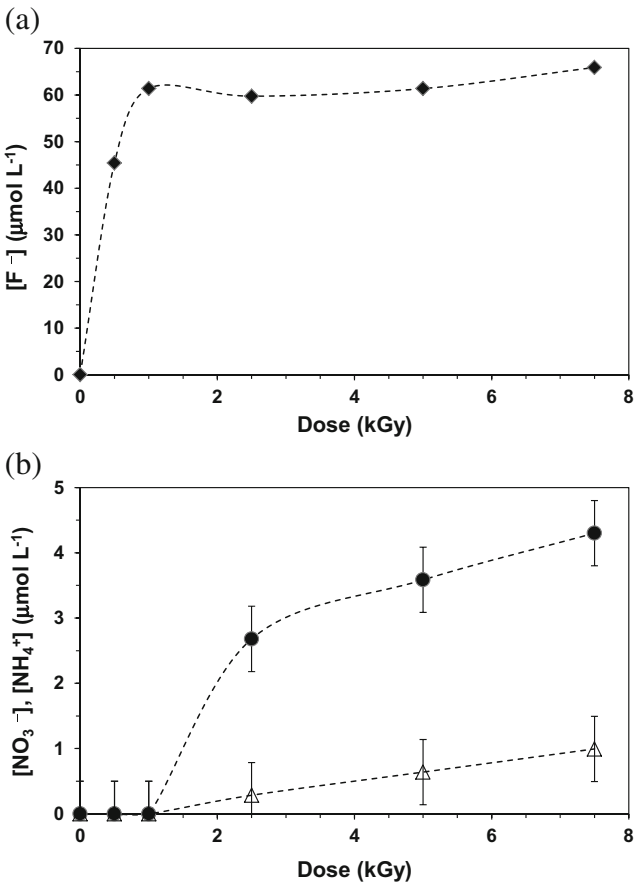

Fig. 2 (**a**) Fluoride concentration (*diamond*) and (**b**) nitrate (*circle*) and ammonium (*triangle*) concentrations for electron beam-irradiated samples at different doses. Initial FLX concentration: $[FLX]_0 = 19.4 \pm 0.2$ mg L^{-1}; initial pH: 6

Proposed degradation pathway of FLX degradation by EBI

The identification of organic byproducts from FLX degradation was performed by direct injection experiments using UHR-QqTOF mass spectrometry. The mass assigned to the $[M+H]^+$ ions of the analytes, in each acquired mass spectrum, was treated as an independent measurement. The elucidation of some peaks in the mass spectrum is shown in Fig. 3; the other peaks remain undefined. A possible degradation pathway of FLX degradation by EBI is proposed (Fig. 4).

The electrophilic addition of hydroxyl radicals to aromatics ring systems constitutes an important route of HO· radical attack on organic pollutants (Batista et al. 2014). FLX molecules ($[M+H]^+$ at *m/z* 310.1587, $C_{17}H_{18}F_3NO$) undergo electrophilic addition of hydroxyl radicals to the aromatic rings, which constitute the initial step that leads to compound P1 with $[M+H]^+$ at *m/z* 326.1547 ($C_{17}H_{18}F_3NO_2$) (Lam et al. 2005). The C–O bond cleavage of compound P1 leads to product P2 [2-(methylamino) ethyl benzyl alcohol] ($[M+H]^+$ at *m/z* 166.1315, $C_{10}H_{15}NO$) and

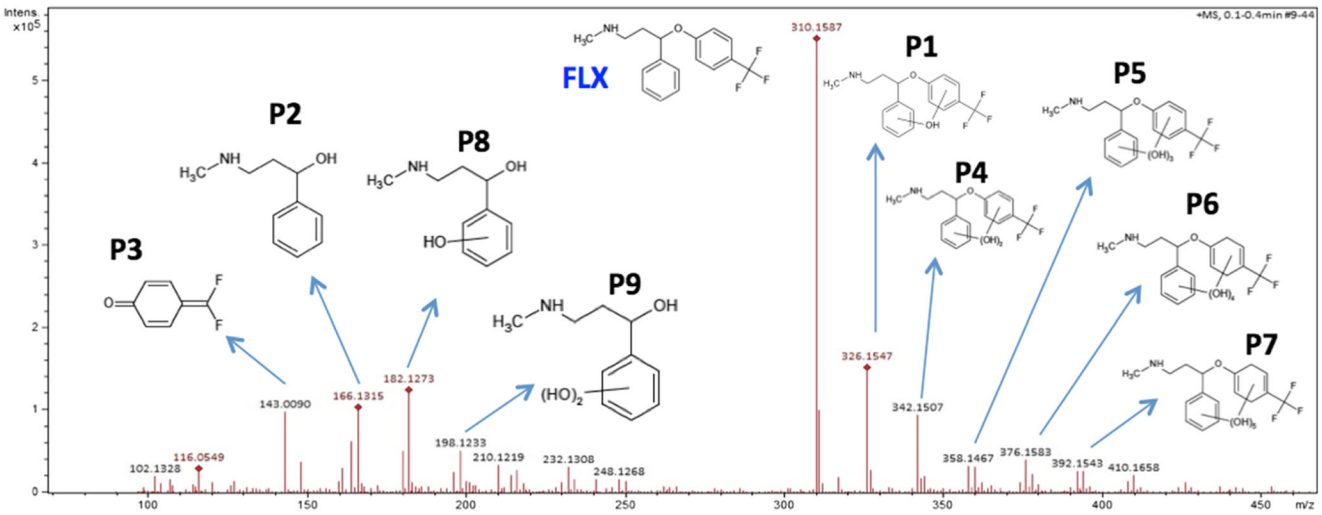

Fig. 3 Time-of-flight mass spectrum obtained after FLX degradation by EBI at 1.0 kGy

to the difluoroquinonoid species P3 [4-(difluoromethylene)-2, 5-cyclohexadiene-1-one] ($[M+H]^+$ at *m/z* 143.0090, $C_7H_4F_2O$). Further hydroxylation of compound P2 leads to compounds P8 ($[M+H]^+$ at *m/z* 182.1273, $C_{10}H_{15}NO_2$) and P9 ($[M+H]^+$ at *m/z* 198.1233, $C_{10}H_{15}NO_3$). The highest intensity peaks corresponding to compounds P1, P2, and P8 permitted to obtain their MS-MS spectra (see Supplementary Information, Fig. S1).

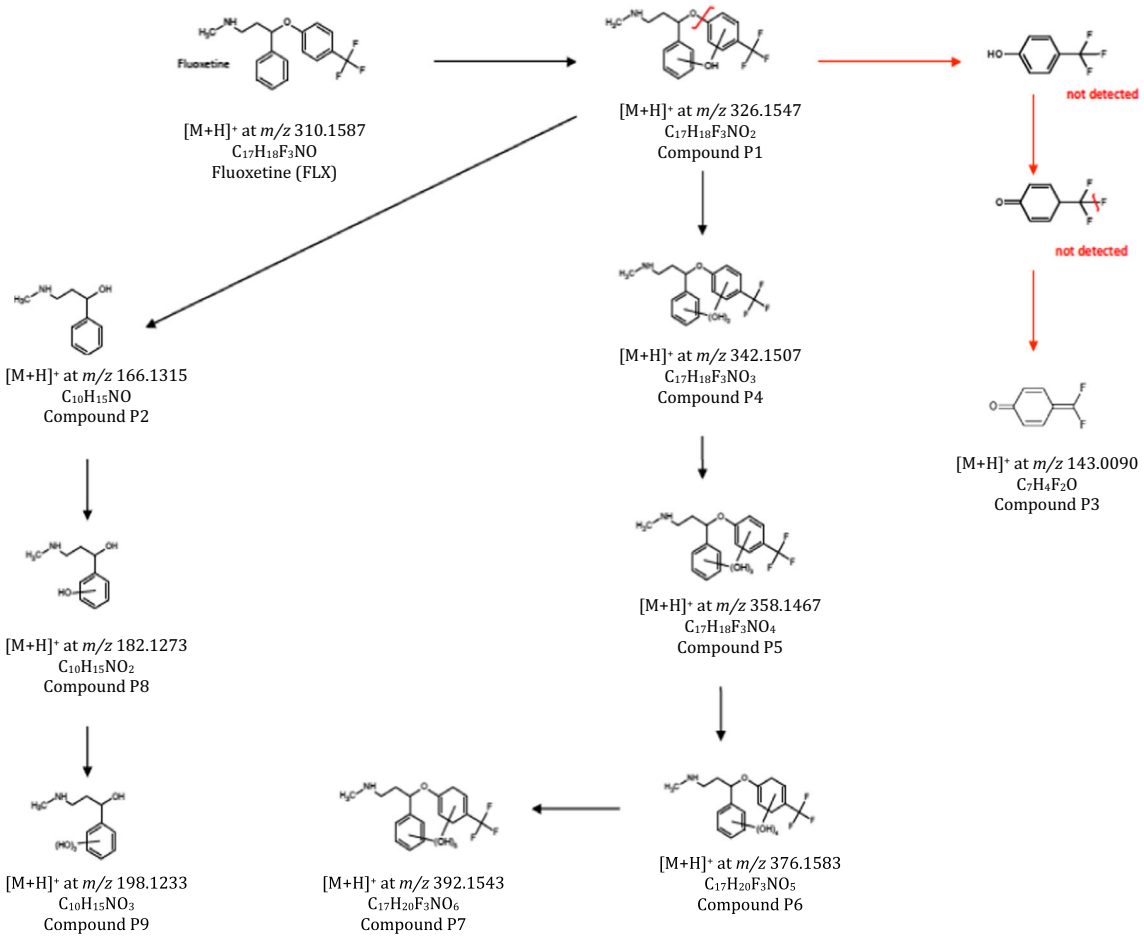

Fig. 4 Proposed degradation pathway of FLX degradation by EBI

Degradation products P2 and P3 had previously been identified during both direct and indirect photolysis of FLX by Lam et al. (2005)), who explained their formation by the O-dealkylation of FLX molecules. Following the pathway rationalized by the authors, the HO· radical (in our case generated from water radiolysis under EBI) adds to the ring with the $-CF_3$ group to give a hydroxycylohexadienyl radical, which is converted into a phenoxyl radical and product P2. The phenoxyl radical is subsequently protonated and the resulting phenolic species hydrolyzes instantaneously to release a fluoride anion, giving product P3 (Lam et al. 2005).

Méndez-Arriaga et al. (2011) identified product P2 during the degradation of FLX by the UV/TiO_2 process in aqueous alkaline medium. As observed by the authors, the formation of poly-hydroxylated species is expected during FLX degradation. In fact, in our study, further hydroxylation of compound P1 led to compounds P4, P5, P6, and P7 identified by UHR-QqTOF mass spectrometry, with $[M+H]^+$ at *m/z* 342.1507 ($C_{17}H_{18}F_3NO_3$), 358.1467 ($C_{17}H_{18}F_3NO_4$), 376.1583 ($C_{17}H_{20}F_3NO_5$), and 392.1543 ($C_{17}H_{20}F_3NO_6$), respectively.

Lam et al. (2005) argue that the amount of fluoride anions found in solution is expected to be 1:1 fluoride:FLX. In fact, the identification of compound P3 with $[M+H]^+$ at *m/z* 143.0090 and the dose-history of fluoride concentration in Fig. 2a supports this mechanism. Contrasting with the experimental observations of Lam et al. (2005) during FLX photodegradation, our results suggest that the hydrolysis of the trifluoromethyl group to a carboxylic acid, with release of three fluoride ions is not expected to occur to an important extent under EBI, regardless of dose used. Furthermore, the well-known dehalogenation reaction of compounds driven by aqueous electrons (e^-_{aq}), initiated by dissociative electron attachment (Cooper et al. 2004) to the $-CF_3$ group could also contribute to the loss of a fluoride ion. However, reducing e^-_{aq} are easily scavenged by H_3O^+ ions in acidic solutions and by oxygen as well, becoming less available for reaction with FLX molecules in comparison with HO· radicals.

Figure 2b reveals that nitrate anions concentration was below the detection limit up to 0.5 kGy and then from 2.5 kGy increased with increasing dose, achieving 4.3 μmol L^{-1} at 7.5 kGy. The concentration of ammonium ions was also below the detection limit up to 1 kGy and then increased gradually to about 1 μmol L^{-1} for 7.5 kGy; at this dose, NO_3^- and NH_4^+ concentrations summed up, representing about 8.5 % of the nitrogen atoms initially existing in FLX molecules. These results suggest that the amine functional group on FLX molecules was poorly mineralized and nitrogen-containing organic substances remained in solution. These results are in agreement with the UHR-QqTOF mass spectrometry measurements and with the FLX degradation pathway we propose.

TOC measurements shown in Fig. 1a reveal incomplete FLX mineralization, with only 16.4 % TOC removal at 7.5 kGy, which is related to the formation of recalcitrant organic byproducts; mineralization was very limited even at higher doses, e.g., 22.2 % at 20 kGy. Incomplete TOC removal during electron beam irradiation was also previously reported for dye molecules in aqueous solution (Abdou et al. 2011; Paul et al. 2011). Homlok et al. (2011) obtained about 30 and 50 % TOC removals at 5 and 20 kGy, respectively, during diclofenac degradation using irradiation technology. By contrast, Méndez-Arriaga et al. (2011) obtained ca. 60–80 % FLX mineralization after 60 min of UV irradiation at different TiO_2 concentrations at pH 11; at pH 5, however, TOC removal was similar to the result we obtained (ca. 20 %). In the present investigation, the UFLC analysis showed the disappearance of FLX (retention time = 5 min), which is converted into organic byproducts with lower retention times (1, 1.3, 1.75, 3.7, and 4.3 min) (see Supplementary Information, Fig. S2). For doses higher than 2.5 kGy, however, the concentration of byproducts was below the detection limit of the chromatographic method, probably owing to the formation of very low or non-fluorescent compounds.

Toxicity measurements

Before irradiation, the acute toxicities of FLX-containing solutions, given by the average effect concentrations that immobilized 50 % of exposed living organisms (EC50%), were 13.2 ± 0.9 % for *D. similis* and 24.6 ± 1.7 % for *V. fischeri*, which correspond to EC50 = 1.32 ± 0.10 mg L^{-1} and 1.23 ± 0.08 mg L^{-1}, respectively; FLX was slightly more toxic to *V. fischeri*. Kwon and Armbrust (2006) reported EC50 values for FLX in the range 0.23–0.51 mg L^{-1} to neonate *Ceriodaphnia dubia*, the second highest toxicity among different selective serotonin reuptake inhibitors (SSRI); Brooks et al. (2003) mentioned EC50 values of 0.82 and 0.234 mg L^{-1} for *Daphnia magna* and *C. dubia*, respectively, for reconstituted hard water-containing FLX. Stanley et al. (2007) measured immobilization and reproduction LOEC (lowest observed effect concentrations) values for the 21-day chronic *D. magna* exposure to *R*-FLX (0.429 mg L^{-1}), *rac*-FLX (0.430 mg L^{-1}), and *S*-FLX (0.444 mg L^{-1}). Boström and Berglund (2015) found EC50 values for FLX to *D. magna* (48 h immobilization tests) of 27 mg L^{-1} (9.2–87), 4.6 (2.6–8.2), and 0.75 (0.44–1.3) at pH 6.0, 7.5, and 9.0, respectively (90 % confidence intervals in parentheses). Data on the toxicity of FLX to *V. fischeri* for aqueous samples have not been previously published for comparison.

Figure 5 shows the apparent acute toxicity (in toxic units, TU = 100/EC50%) of samples irradiated at 5 and 20 kGy in comparison with non-irradiated samples. These results suggest that the organic byproducts formed following the hydroxylation of FLX molecules, which are associated with the

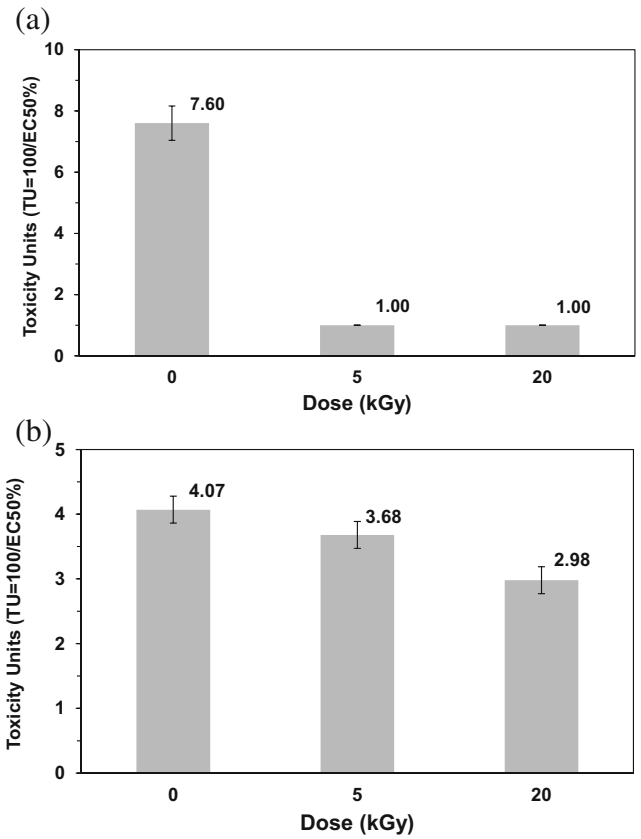

Fig. 5 Acute toxicity (in toxic units, TU = 100/EC50%) for electron beam-irradiated samples at different doses obtained for the test-organisms (**a**) *D. similis* and (**b**) *V. fischeri*

remaining TOC, do not increase the acute toxicity to *D. similis* and *V. fischeri*. The toxicity removal after EBI exposure at 5 kGy was higher for *D. similis* than for *V. fischeri*, with 86.8 and 9.6 % decreases in toxicity units (TU), respectively. The parent antidepressant seems to be the main responsible to induce toxic effects on *D. similis*, with a large decrease in TU following the removal of FLX below the detection limit at 5 kGy, despite the corresponding TOC removal of only 12.5 %. In contrast, the small decreases in TU values following irradiation at 5 kGy (9.6 %) and 20 kGy (26.8 %) suggest that the transformation by-products are toxic to *V. fischeri*.

Nine degradation byproducts from EBI-driven FLX degradation were elucidated using mass spectrometry, as discussed in Section 3.2. Further investigation is required to correlate these compounds with the toxicity bio-assays results.

Finally, EBI at a dose of 5 kGy was applied to an aqueous solution of FLX diluted 50 % *v*/*v* in raw domestic sewage (to give the same FLX concentration of undiluted solutions), resulting in 80.0 and 22.2 % decreases in acute toxicity for *D. similis* and *V. fischeri*, respectively. This suggests that the performance of EBI for toxicity removal seems not to depend on the characteristics of the water matrix under treatment.

Conclusions

The results obtained in this study demonstrate the feasibility of electron beam irradiation (EBI) to remove the antidepressant fluoxetine (FLX) (Prozac®) from water, with more than 90 % FLX removed at 0.5 kGy and virtually complete removal at doses higher than 2.5 kGy (with [FLX] below the detection limit of the chromatographic method, i.e., 0.012 mg L^{-1}). Complete mineralization was not observed even at 20 kGy, with only 22.2 % TOC removal. The decreases in acute toxicity achieved 86.8 and 9.6 % for *D. similis* and *V. fischeri* after EBI exposure at 5 kGy, respectively. This is a good indicator of the ability of EBI regarding the removal of residual toxicity from wastewaters containing FLX. It is worth noting that the parent antidepressant seems to be the main responsible to induce toxic effects on *D. similis*, while toxicity of the transformation byproducts is expected for *V. fischeri*.

Nine transformation products were elucidated during EBI-driven FLX degradation using direct injection mass spectrometry. Our proposed degradation pathway includes the electrophilic addition of hydroxyl radicals generated from water radiolysis under EBI to the aromatic groups, further hydroxylation of ring systems, and also release of fluoride anions. About one third of the carbon bound fluorine atoms originally present in FLX molecules were released to the solution as F^- ions for doses higher than 1 kGy. In contrast, the total amount of NO_3^- and NH_4^+ ions formed indicate the mineralization of only 8.5 % of the carbon-bound nitrogen atoms, at a dose of 7.5 kGy.

The experimental results provided information on accurate masses and might be included in future studies devoted to the elucidation of byproducts generated from FLX degradation. Nevertheless, further detailed mass spectrometry studies are needed to correlate the proposed FLX degradation mechanism with toxicity measurements. In conclusion, our results suggest that EBI could be an alternative to eliminate FLX and to decrease residual toxicity from wastewater generated in pharmaceutical formulation facilities, although energy consumption and cost per treated cubic meter should be considered.

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