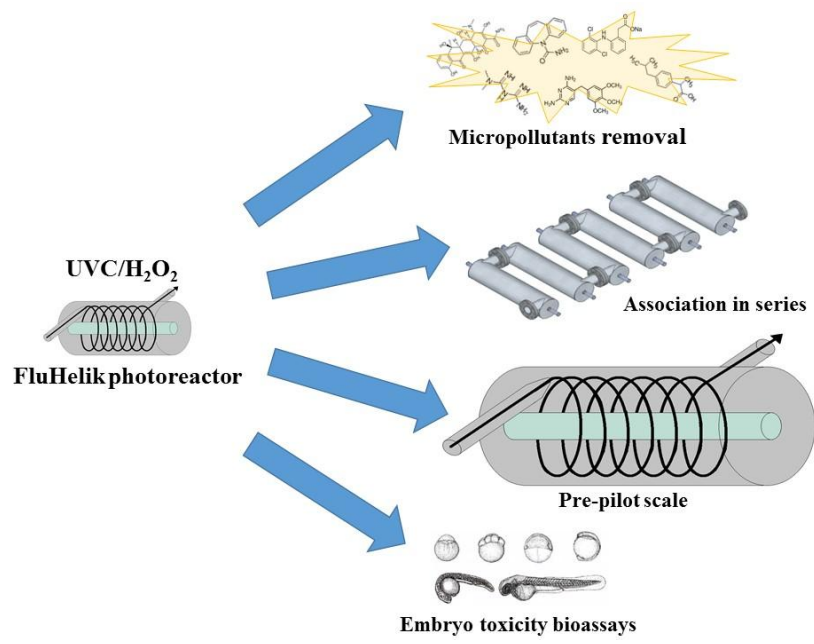


Graphical Abstract



Highlights

- The FluHelik photoreactor proved to enhance the OTC oxidation by UVC/H₂O₂;
- The FluHelik design showed superior performance than conventional Jets photoreactor;
- FluHelik reactor + UVC/H₂O₂ effectively reduced CECs complying with Swiss legislation;
- FluHelik reactor + UVC/H₂O₂ effectively reduced CECs toxicity to zebrafish embryos;
- The FluHelik scale-up proved to be feasible employing several reactors in series.

27 **Abstract**

28 An innovative photoreactor, FluHelik, was used to promote the degradation of
29 contaminants of emerging concern (CECs) by a photochemical UVC/H₂O₂ process.

30 First, the system was optimized for the oxidation of a model antibiotic, oxytetracycline
31 (OTC), using both ultrapure water (UPW) and a real urban wastewater (UWW)
32 (collected after secondary treatment) as solution matrices. Following, the process was
33 evaluated for the treatment of a UWW spiked with a mixture of OTC and 10 different
34 pharmaceuticals established by the Swiss legislation at residual concentrations (Σ CECs
35 $< 660 \mu\text{g L}^{-1}$). The performance of the FluHelik reactor was analyzed both at lab and
36 pre-pilot scale in multiple and single pass flow modes.

37 The efficiency of the FluHelik photoreactor, at lab-scale, was evaluated at different
38 operational conditions (H₂O₂ concentration, UVC lamp power (4, 6 and 11 W) and flow
39 rate) and further compared with a conventional Jets photoreactor. Both photoreactors
40 exhibited similar OTC removal efficiencies at the best conditions; however, the
41 FluHelik reactor showed to be more efficient (1.3 times) in terms of mineralization
42 when compared with the Jets reactor. Additionally, the efficiency of the UVC/H₂O₂
43 photochemical system using the FluHelik photoreactor in reducing the toxicity of the
44 real effluent containing 11 pharmaceuticals was evaluated through zebrafish (*Danio*
45 *rerio*) embryo toxicity bioassays. FluHelik scale-up from laboratory to pre-pilot to
46 promote UVC/H₂O₂ photochemical process proved to be feasible.

47

48 **Keywords:** FluHelik photoreactor; UVC/H₂O₂; CECs; Urban wastewater; Zebrafish
49 embryo toxicity test.

50 **Introduction**

51 UVC/H₂O₂ photochemical process is based in the homolytic cleavage of H₂O₂
52 molecules by UVC light, resulting in highly reactive species (HO[•]), able to eliminate an
53 extensive variety of pollutants from water. However, due to the low values of molar
54 absorption coefficient of H₂O₂ at 254 nm, high H₂O₂ or UV dose is required to achieve
55 an efficient performance (Krishnan et al., 2017). On the other hand, urban wastewaters
56 composition can reduce significantly the system efficiency due to the presence of light
57 absorbing species (NOM, nitrite, etc.) (Diya'uddeen et al., 2011). The efficiency of
58 UVC/H₂O₂ photochemical process is also largely influenced by the reactor
59 hydrodynamics regime, which must promote an uniform UV fluence within the reactor
60 (Cambié et al., 2016). Here enters the importance of the reaction mixing conditions for
61 the treatment effectiveness (Karpel Vellleitner et al., 1997). Several commercial
62 reactors incorporate different mixing systems, such as static mixers and conical
63 dispersion components, to improve the degree of mixing inside the reactor, promoting
64 the contact between reagents/pollutants and the emitted UVC photons. Normally, the
65 irradiation source is located in the most turbulent zone of the reactor (Masschelein,
66 1992). Nowadays there is a great variety of photoreactors with diverse geometries
67 leading to different hydrodynamics. Generally, the photoreactor comprises a cylindrical
68 shell of stainless steel housing a concentric quartz sleeve filled with an UVC lamp and
69 the water to be treated flows between the concentric tubes (annular reactor). However,
70 the irradiation source may be also external, such as the parallel plate reactors or
71 cylindrical reactors reported by Noël (2017)). The mixing and irradiation conditions can
72 also be improved through introduction of a multi-lamp design (Boyjoo et al., 2014) or
73 by usage of rotating annular reactors (Subramanian and Kannan, 2010) or spinning disc
74 reactors (Yatmaz et al., 2001). Therefore, even with the same lamp type and intensity,

75 reagents/pollutants dosages and similar flow rates, the photons dissemination as well
76 and the pollutants removal may be completely different (Caris, 2011). Although
77 photochemical reactors have been already applied in water/wastewater treatment plants,
78 the process is not widely disseminated because of the inherent limitations that it
79 presents, namely in terms of energy costs and efficiency. Therefore, breakthrough
80 designs for photoreactors are required to achieve a cost-effective treatment solution (Su
81 et al., 2014).

82 The present work focuses on the application of an innovative photoreactor, FluHelik, in
83 the removal of contaminants of emerging concern (CECs) from urban wastewaters, as a
84 polishing step, using a UVC/H₂O₂ photochemical process. The FluHelik photoreactor
85 comprises a cylindrical shell of stainless steel, internally polished, with inlet and outlet
86 pipes located perpendicularly to the fluid flow and tangentially to the shell in horizontal
87 plane and at the top in opposite sides. A concentric inner quartz sleeve houses an UVC
88 lamp. This configuration induces unique fluid dynamics (high degree of mixing) and
89 irradiation properties (a more homogeneous UV radiation distribution) by promoting a
90 helical motion of the fluid around the UVC lamp. First, synthetic solutions of OTC or
91 UWW fortified with OTC were used as reaction matrices. Two configurations of
92 photoreactors were employed: FluHelik and Jets (four inlet and four outlet pipes placed
93 in parallel with the fluid flow direction at the ends of the tube). Process efficiency was
94 evaluated as a function of several operational conditions, namely: (i) recirculation flow
95 rate, (ii) H₂O₂ concentration and (iii) UVC lamp power. In addition, the feasibility of
96 implementing the FluHelik reactor for the UVC/H₂O₂ process was tested both at
97 laboratory and at pre-pilot scale either in multiple or single pass flow mode. Finally, the
98 treatment of a real urban wastewater matrix spiked with a mixture of 11 CECs, at

99 residual concentrations, was evaluated using the FluHelik photoreactor and the
100 UVC/H₂O₂ system.

101 Considering that oxidation by-products might be more toxic and/or persistent than the
102 parent compounds, toxicological studies are needed to determine their deleterious
103 effects on ecosystems and human health. Zebrafish (*Danio rerio*) have been widely used
104 in Fish Embryo Toxicity (FET) Tests to assess the toxicity of several priority pollutants.
105 This embryonic bioassay has high sensitivity and low cost. Furthermore, zebrafish
106 embryos are translucent which allows for the monitoring of embryo development under
107 a stereomicroscope (Macedo et al., 2017; Zhang et al., 2015). This bioassay has recently
108 been proposed by the OECD as an alternative to classical acute fish toxicity tests
109 (Lammer et al., 2009), and is an appropriate tool to assess the decrease in toxicity after
110 treatment of wastewaters contaminated by emerging pollutants.

111 In this sense, embryo toxicity bioassays with zebrafish (*Danio rerio*) were used to
112 evaluate the initial effluent toxicity and possible attenuation of the toxic effect after the
113 UVC/H₂O₂ treatment by using the FluHelik reactor.

114 **2. Materials and methods**

115 *2.1 Chemicals*

116 Oxytetracycline hydrochloride (OTC, C₂₂H₂₄N₂O₉.HCl, 496.89 g/mol) was supplied by
117 Sigma-Aldrich and used as a model compound. Hydrogen peroxide (Fisher Chemical,
118 purity 49.5% (w/v)) was used as oxidant. Na₂SO₃ in a Na₂SO₃-to-H₂O₂ molar ratio of
119 1:1 was added to CECs and dissolved organic carbon (DOC) samples for H₂O₂
120 elimination (Jeong et al., 2010). Catalase (Sigma-Aldrich) was added to the samples to
121 eliminate residual H₂O₂ before performing ecotoxicological quality tests. Ammonium
122 monovanadate (Merck, p. a.) was used as colorimetric reagent to determine H₂O₂
123 concentration. Sulfuric acid (Pronalab, purity 96%, 1.84 g/cm³) and sodium hydroxide

124 (Merck) were used for pH adjustment. Ultrapure water was obtained from a Millipore®
125 Direct-Q system (18.2 MΩ cm resistivity at 25 °C). Real wastewater sample was
126 collected downstream from the secondary treatment of an urban WWTP from Northern
127 Portugal in September 2017. Its physicochemical characteristics, including the CECs
128 residual concentrations detected in the raw effluent, are summarized in Table 1. The
129 ultrapure water and the secondary effluent both spiked with 20 mg OTC L⁻¹ were used
130 as feed solutions. Table 2 shows the 11 pharmaceutical compounds added to the real
131 wastewater. Tricaine (1000 mg g⁻¹) used to anesthetize zebrafish larvae was purchased
132 from Pharmaq. Sodium hydrogen carbonate used as a buffer in the preparation of the
133 anesthetic was supplied by Merck KGaA. All the other chemicals supplied by VWR-
134 Prolabo, Sigma-Aldrich, Panreac, Merck, Fisher Scientific and Pronalab were either of
135 HPLC grade or analytical grade.

136 **Insert Table 1**

137 **Insert Table 2**

138 *2.2 Analytical determinations*

139 OTC concentration was followed by HPLC using a VWR Hitachi ELITE LaChrom LC
140 fitted with a Merck LiChrosorb® RP-18 (5 μm) LiChroCART® 125-4 column at 25 °C
141 and a diode array detector (DAD). Low-molecular-weight carboxylic acids (LMWCA)
142 concentrations were determined by ion-exclusion HPLC using the VWR Hitachi ELITE
143 LaChrom LC fitted with a Phenomenex Rezex™ ROA-Organic Acid H+ (8%) 300
144 mm × 7.8 mm column at room temperature (25 °C). A detailed description of OTC and
145 LMWCA analysis is given in Supplementary Material.

146 H₂O₂ concentration was determined by the colorimetric (λ = 450 nm) metavanadate
147 method (Nogueira et al., 2005). Dissolved organic carbon (DOC), chemical oxygen
148 demand (COD), total dissolved nitrogen, total dissolved iron, total suspended solids

149 (TSS), volatile suspended solids (VSS), total phosphorous, pH, temperature and
150 turbidity, as well as inorganic anions and cations concentrations were assessed
151 according to the procedures already described by [Moreira et al. \(2016\)](#)). Conductivity,
152 dissolved oxygen and redox potential were determined by a HANNA Instruments HI
153 9828 Multiparameter meter.

154 CECs determination in water samples, at residual concentrations, was performed in an
155 Acquity UPLC® liquid chromatograph system from Waters (Milford, MA, USA). A
156 sample volume of 45 µL was directly injected into a Luna C18 100A column (50 mm ×
157 2 mm, 3µm particle size) supplied by Phenomenex (Torrance, CA, USA) maintained at
158 a constant temperature of 30 °C. The target compounds were separated at a flow rate of
159 0.2 mL min⁻¹ using 0.1% of formic acid in both, Milli-Q water (A) and MeOH (B) as
160 eluents. The applied gradient was as follows: 0–1 min, 0% B; 1–8 min, linear gradient
161 to 100% B; 7–13 min, 100% B and finally 13–20 min, 0% B. The system was interfaced
162 to a XEVO TQD® triple quadrupole mass spectrometer equipped with an electrospray
163 interface (ESI). Nitrogen was used as a nebulizing and drying gas and Argon was used
164 as collision gas. The analytes were determined in the electrospray (positive and negative
165 polarities) and multiple-reaction monitoring (MRM) mode of acquisition. Two MRM
166 transitions were used as quantifier and qualifier for each compound (see Table S1 for
167 detailed information). The method assured limits of quantification (LOQ) between 10
168 and 100 ng L⁻¹ for all the compounds except for azytromycin (LOQ 1.8 µg L⁻¹), see
169 supplementary material Table S1. Quantification was performed by the matrix matched
170 calibration method using standards prepared in treated wastewater in the 1-100 µg L⁻¹
171 (2-100 µg L⁻¹ in the case of azytromycin) range (which was checked to be linear,
172 R²>0.99 for all the studied analytes). Repeatability of the determination was checked in

173 terms of relative standard deviation (RSD) at $10 \mu\text{g L}^{-1}$ level and the values were lower
174 than 10 % for all compounds.

175 *2.3 Experimental apparatus*

176 *2.3.1 Lab-scale*

177 The lab-scale system consists of: (i) a FluHelik stainless steel reactor or a Jets glass
178 reactor; (ii) a 1.5 or 5.5 L recirculation cylindrical glass vessel coupled to a thermostatic
179 bath (Julabo, model F12-EH) under magnetic stirring at 400 rpm (Velp Scientifica,
180 model T.ARE); (iii) a gear pump (Ismatec, model BVP-Z) to promote the fluid
181 recirculation. The system units were connected by polytetrafluoroethylene (PTFE)
182 tubing. Three low pressure mercury UVC lamps were used: (i) a 4 W power Philips
183 TUV G4T5, (ii) a 6 W power Philips TUV G6T5, and (iii) a 11 W power Philips TUV
184 G11T5. The photon flow for each photoreactor and respective UVC lamp power was
185 determined by H_2O_2 (73.5 mM) actinometry ([Kuhn et al., 2004](#)).

186 *2.3.1.1 Jets photoreactor*

187 The Jets reactor ($V_{\text{illuminated}} = 553 \text{ mL}$; light path-length = 40 mm) comprises (i) a
188 borosilicate glass cylindrical tube ($d_{\text{int}} = 66 \text{ mm}$; length = 184 mm; thickness = 1.8 mm)
189 with four inlets and four outlets placed in parallel with the fluid flow at the ends of the
190 tube, and (ii) a concentric inner quartz tube ($d_{\text{ext}} = 23 \text{ mm}$; length = 184 mm; thickness =
191 1 mm) filled with an UVC lamp. A scheme of the Jets photoreactor can be seen
192 elsewhere ([Soares et al., 2016](#)). Photonic fluxes of 0.8, 2.0 and $2.4 \text{ J}_{\text{UV}} \text{ s}^{-1}$ were
193 determined for 4, 6 and 11 W UVC lamps, respectively.

194 *2.3.1.2 FluHelik photoreactor*

195 The FluHelik reactor is an annular channel reactor consisting of (i) a cylindrical shell of
196 stainless steel ($d_{\text{int}} = 72 \text{ mm}$; length = 186 mm; thickness = 2 mm), internally polished,
197 with inlet and outlet pipes located perpendicularly to the fluid flow and tangentially to

198 the shell in horizontal plane and at the top in opposite sides, and (ii) a concentric inner
199 quartz tube ($d_{ext} = 23$ mm; length = 186 mm; thickness = 1 mm) housing an UVC lamp
200 ($V_{illuminated} = 680$ mL; light path-length = 46.0 mm). Fig. 1 displays the structure of this
201 reactor. The entire experimental unit was already fully described by [Moreira et al.](#)
202 ([2019](#)). Photonic flow of $2.0 \text{ J}_{UV} \text{ s}^{-1}$ was determined for the 6 W UVC lamp.

203 **Insert Figure 1**

204 *2.3.2 Pre-pilot scale - FluHelik*

205 The pre-pilot scale system consists of: (i) a FluHelik photoreactor made of a stainless
206 steel cylindrical shell ($d_{int} = 154$ mm; length = 480 mm; thickness = 7 mm), internally
207 polished, with tangential inlet and outlet; (ii) a concentric quartz tube ($d_{out} = 49$ mm;
208 length = 480 mm; thickness = 2 mm) to house a 95 W power Strahler UL C 2G11 UVC
209 lamp ($19.3 \pm 0.3 \text{ J}_{UV} \text{ s}^{-1}$; light path-length = 101.1 mm; $V_{illuminated} = 8.0$ L); and (iii) a
210 cylindrical recirculation tank with 120 L capacity. The various components of the
211 system were connected by polypropylene (PP) tubing. The solution circulates
212 continuously by means of a centrifugal pump (GemmeCotti, model HTM15PP) at a
213 flow rate of 7500 L h^{-1} ($Re = 15000$) regulated by a rotameter.

214 *2.4 Experimental procedure*

215 *2.4.1 Assessment of OTC degradation*

216 A solution consisting of 5 or 20 mg L^{-1} of OTC in ultrapure water or urban wastewater
217 was added to a cylindrical glass vessel and homogenized by recirculation through all the
218 system during 10 min in the darkness. The temperature controller was switched on at a
219 temperature set-point that allowed preserving the inner solution at $25 \text{ }^\circ\text{C}$. A first control
220 sample was taken and then, the hydrogen peroxide was added and the UVC lamp was
221 switched on, stating the reaction beginning. Samples were taken at different time
222 intervals to evaluate the oxidation process. The OTC oxidation was evaluated using

223 different reaction conditions: i) direct photolysis (absence of oxidant), ii) only with
 224 H₂O₂ (absence of radiation), iii) UVC/H₂O₂. The efficiency of the process was analyzed
 225 by changing the H₂O₂ initial dosage (20-700 mg L⁻¹), the flow rate (50-100 L h⁻¹) and
 226 the UVC lamp intensity (4, 6 or 11 W) at lab-scale and the H₂O₂ dosage (100-700 mg L⁻¹)
 227 at pre-pilot scale. Both reactors (FluHelik and Jets) were operated in single pass and
 228 in multiple pass flow mode. In single pass flow mode, the solution to be treated was
 229 added to the glass vessel, followed by the hydrogen peroxide addition and homogenized
 230 during 2 hours in the dark. A first control sample was taken and then the radiation
 231 source was switched on, starting the reaction. Samples were collected at different time
 232 periods until reaching the stationary phase. Immediately after samples collection,
 233 Na₂SO₃ in a Na₂SO₃:H₂O₂ molar ratio of 1:1 was added to quench H₂O₂.
 234 The photochemical space time yield (PSTY, m³_{water} m⁻³_{reactor} day⁻¹ kW⁻¹) was calculated
 235 via Eq. (1) (Leblebici et al., 2015).

$$236 \text{ PSTY} = \frac{\text{STY}}{\text{LP}} \quad (1)$$

237 Where the space-time yield (STY, m³_{water} m⁻³_{reactor} day⁻¹) is standardized to the volume
 238 (m³) of the wastewater processed from 20 mg L⁻¹ to 0.02 mg L⁻¹ of OTC (three orders of
 239 magnitude) in a 1 day by the reactor when it is scaled up to 1 m³ (V_R). STY can be
 240 calculated from the apparent reaction rate *k* (day⁻¹), considering the FluHelik reactor
 241 operated in a loop as a continuous stirred tank reactor (Eq. 3).

$$242 C_A = \frac{C_{A_0}}{1 + k\tau} \quad (2)$$

243 where *C_A* is the outlet concentration in mg L⁻¹, *C_{A0}* is the inlet concentration in mg L⁻¹
 244 and *τ* is the passage time in days. From Eq. 3, *τ* can be calculated and used to determine
 245 the STY, according to Eq. 4.

$$246 \text{ STY} = \frac{V_R}{\tau} = \frac{1\text{m}^3}{\tau} = \frac{k}{999} \quad (3)$$

247 In its turn, LP (kW) is the standardized lamp power, which would illuminate 1 m^3 of the
248 reactor, where the lamp power (P) in kW is normalized to the volume (V , m^3) of the
249 reaction medium in the experimental setup (Eq. 4).

$$250 \quad LP = P \frac{1\text{m}^3}{V} \quad (4)$$

251 *2.4.2 Assessment of CECs degradation*

252 An urban wastewater fortified with $60 \mu\text{g L}^{-1}$ of OTC and 10 additional CECs (present
253 in Table 2) from the 12 established by the Swiss legislation (Hochstrat et al., 2015) was
254 added to a cylindrical glass vessel and homogenized by recirculation through all the
255 system during 10 min in the darkness. The temperature controller was switched on at a
256 temperature set-point that allowed preserving the inner solution at $25 \text{ }^\circ\text{C}$. A first control
257 sample was taken and then, the hydrogen peroxide was added and the 6 W UVC lamp
258 was switched on, stating the reaction beginning. Samples were taken after 30 min of
259 reaction to evaluate the oxidation process. The efficiency of the process was analyzed
260 by changing the H_2O_2 initial dosage ($10\text{-}500 \text{ mg L}^{-1}$) using the lab-scale FluHelik
261 photoreactor at a flow rate of 100 L h^{-1} and pH 7.5. The system was operated in single
262 pass and in multiple pass flow mode. Immediately after samples collection, catalase
263 solution was added to quench H_2O_2 .

264 *2.5 Toxicity screening with zebrafish embryo bioassays*

265 Zebrafish embryo bioassays were used to evaluate the efficiency of the UVC/ H_2O_2
266 photochemical system with the FluHelik photoreactor in reducing the toxicity of the
267 UWW spiked with 11 CECs (Table 1 and 2). The bioassays included the exposition of
268 zebrafish embryos to the UWW spiked with trace level of 11 CECs before (UWW + 11
269 CECs) and after treatment (UWW + 11 CECs + Treatment). The toxicity of the UWW
270 without the CECs addition (UWW) was also assessed.

271 *2.5.1 Fertilization and embryos collection*

272 A stock of adults zebrafish were maintained in 150 L aquarium with dechlorinated
273 filtered and aerated water at 28 ± 1 °C, under a photoperiod of 14:10 h (light:dark). The
274 animals were fed two times per day with commercial fish diet Tetramin (Tetra, Melle,
275 Germany) supplemented with *Artemia spp* (Barros et al., 2018). For the zebrafish
276 reproduction, in the afternoon before breeding, a group of males and females (2:1) was
277 isolated in a breeding box under the same water and photoperiod conditions as the
278 stock. At the following day, 1.5 h after the beginning of the light period the eggs were
279 collected and cleaned to be used in the zebrafish embryo bioassays (Barros et al., 2018;
280 Ribeiro et al., 2015; Torres et al., 2016).

281 2.5.2 Zebrafish embryo bioassays

282 The bioassays were carried out with slight modification of OECD Fish Embryo Acute
283 Toxicity (FET) Test 236 (Barros et al., 2018; OECD, 2013). After observation in a
284 magnifying glass, cleaned fertilized embryos were randomly allocated into 24-wells
285 plates (one embryo per well) filled with 2 mL of freshly solutions and control. The
286 experiments consisted of 5 treatments of 20 embryos each divided in six replicates:
287 UWW, UWW + 11 CECs, UWW + 11 CECs + Treatment and Control (dechlorinated
288 water). In each 24-well plate was allocated one treatment (20 embryos) plus an internal
289 plate control (4 embryos) (Fig. S1).

290 The 24-well plates were randomly maintained on a water bath at 26.5 ± 0.5 °C for 80 h.
291 Embryos were checked, every day, for mortality and dead embryos were removed. The
292 medium was renewed daily in order to maintain oxygen and the integrity of the
293 solutions. At the end of the bioassay (80 hpf), under an inverted microscope (Nikon
294 Eclipse 5100T) equipped with a digital camera (Nikon D5-Fi2), morphological
295 abnormalities on tail or yolk-sac; pericardial oedema and lordosis were recorded as
296 present or absent (Fig. S2) (Barros et al., 2018). The different abnormalities' were

297 grouped and presented as total abnormalities. At the same time the length and the yolk
298 sac perimeter of 10 larvae per treatment were recorded.

299 *2.5.3 Statistical analysis*

300 Data were first checked for homogeneity of variances (Levene's test) and subsequently
301 analyzed by one-way ANOVA followed by Fisher's least significant difference test
302 (LSD) or nonparametric analysis (Kruskall-Wallis ANOVA by ranks followed by
303 multiple comparisons of mean ranks). The significance threshold was set at $p < 0.05$.
304 All statistics were computed with Statistica (Stat-soft, USA).

305 **3. Results and discussion**

306 *3.1 OTC degradation by an UVC/H₂O₂ photochemical system using a conventional Jets* 307 *photoreactor in multiple pass flow mode*

308 Initially it was assessed the effect of pH on the direct photolysis of 5 mg L⁻¹ of OTC
309 with an UVC lamp of 6 W. OTC removals of 82 and 88% after 120 min were achieved
310 at pH 4.5 and 7.5, respectively, without pH control during the experiments. No
311 significant pH variations were observed for the experiments performed at OTC solution
312 pH (4.5). However, the solution pH decreased to values below 6.5 during the
313 experiments performed at an initial pH of 7.5. On the other hand, the OTC removal
314 increased to 98% only after 60 min when the solution pH was controlled at 7.5 through
315 the addition of NaOH solution. For pH values higher than 6.5, the predominant OTC
316 species are negative, which are more susceptible to photochemical degradation ([Liu et](#)
317 [al., 2015](#)).

318 In order to follow the reaction kinetics in a more rigorously way, a synthetic solution
319 with 20 mg OTC L⁻¹ was used in the next experiments. OTC removals above 90% were
320 achieved after 120 and 90 min of reaction under direct photolysis using a 4 and 6 or 11
321 W UVC lamp, respectively. On the other hand, the OTC removal proved also to be

322 possible by H₂O₂ in the absence of radiation, attaining degradations of 20, 30 and 50%
323 after 180 min of reaction using 100, 300 and 500 mg L⁻¹ of oxidant, respectively. As
324 expected, the combination of UVC light with H₂O₂ improved the OTC degradation
325 under all the studied irradiation intensities (Fig. 2). It is noteworthy that the
326 photochemical oxidation of OTC showed to follow a pseudo-first order kinetic model.
327 For all the UVC lamp intensities evaluated, the OTC removal rates increased with the
328 initial oxidant dose, attaining the highest removal rates (see pseudo-first order kinetic
329 constants, *k*, in Table 3) with 100 mg L⁻¹ when using the 4 or the 6 W lamps (Fig. 2a
330 and 2b, respectively) and with 500 mg L⁻¹ of H₂O₂ when the 11 W UVC lamp (Fig. 2c)
331 was employed (within the tested concentrations range). These results suggest an
332 increasing production of hydroxyl radicals ([•]OH) for growing H₂O₂ initial contents (H.
333 Baxendale and A. Wilson, 1957). For higher oxidant dosages, the reaction rates
334 remained constant and, for the 11 W lamp there was even a decrease of 1.3 times in the
335 kinetic constant when using 700 mg L⁻¹ of H₂O₂ (Table 3). In fact, the H₂O₂ in excess
336 can act as an hydroxyl radicals scavenger (Muruganandham and Swaminathan, 2004).
337 This is supported by the growing H₂O₂ consumption for rising initial H₂O₂ doses (Fig.
338 2).

339 **Insert Figure 2**

340 **Insert Table 3**

341 Comparing the OTC removals by the UVC/H₂O₂ system under the best conditions for
342 each of the lamp powers studied (Fig. 2d), no significant differences between the
343 reaction rates (in terms of energy) were observed. For all the systems, an OTC removal
344 above 90% is already achieved with 0.4 kJ L⁻¹. On the other hand, a different behaviour
345 was observed in relation to the OTC mineralization: DOC decays of 13, 50 and 45%
346 after 60 min of reaction were observed at the best conditions when using the 4, 6 and 11

347 W lamps, respectively. It is worth mentioning that, during this reaction period, similar
348 oxidant consumptions were noticed for the 4 and 6 W lamps (c.a. 35 mg H₂O₂ L⁻¹);
349 however, when using the 11 W lamp a 5-fold increase in hydrogen peroxide
350 consumption (higher initial H₂O₂ dose) was observed. These results prove that the
351 higher H₂O₂ consumption associated to the 11 W lamp is not related with a higher
352 mineralization, but probably to parasite reactions. Therefore, the 6 W lamp provided the
353 most suitable photon flow for the experimental set-up used: the 4 W lamp showed to not
354 supply the necessary UV dosage and, in turn, using the 11 W UVC lamp a possible loss
355 of the emitted photons is occurring, probably due to the low molar absorption
356 coefficient of H₂O₂ at 254 nm, requiring higher amounts of H₂O₂ to absorb all those
357 photons.

358 As above-mentioned, although the total degradation of the parent compound (OTC) is
359 achieved in short reaction times, relatively low mineralization is observed. Actually, for
360 longer reaction times (360 min), a mineralization of 62% was reached (6 W UVC lamp;
361 100 mg H₂O₂ L⁻¹), consuming 87 mg L⁻¹ hydrogen peroxide, a higher value than the one
362 predicted by the reaction stoichiometry to completely mineralize 20 mg L⁻¹ of OTC (77
363 mg H₂O₂ L⁻¹). In fact, 38% of the residual DOC corresponds to low-molecular-weight
364 carboxylic acids (LMWCA) in solution, namely oxalic and oxamic acids.

365 The H₂O₂/UVC process led to higher OTC degradation rates with the increment of Q
366 from 50 to 100 L h⁻¹ (Table 3), indicating a change in the hydrodynamic conditions
367 inside the photoreactor. A Re number of 446 ($Q = 100$ L h⁻¹) allowed to a 2.3-fold
368 increase on OTC oxidation rate comparing with a Re of 223 ($Q = 50$ L h⁻¹). At those
369 conditions, an OTC removal above 90% is achieved after 5 min of reaction with 0.4 kJ
370 L⁻¹.

371 *3.2 OTC degradation by an UVC/H₂O₂ photochemical system using an innovative*
372 *FluHelik photoreactor in multiple pass flow mode*

373 An initial H₂O₂ dose of 300 mg L⁻¹ led to a maximum OTC oxidation rate and
374 mineralization, corresponding to a 9-fold increment on reaction rate when compared to
375 direct photolysis (Table 3). A higher oxidant dose (400 mg L⁻¹) led to a slightly decrease
376 in the OTC oxidation rate, due to hydroxyl radicals quenching by the hydrogen peroxide
377 molecule itself. This is also supported by the growing H₂O₂ consumption for rising
378 initial H₂O₂ doses (Fig. 3). Using 300 mg L⁻¹ of oxidant, a 77% mineralization was
379 attained after 360 min with a final residual H₂O₂ concentration of 17 mg L⁻¹. 50% of the
380 remaining DOC was from oxalic and oxamic acids. In turn, the nitrogen content of the
381 OTC compound was converted to nitrites, nitrates and ammonium, with ammonia
382 representing the largest fraction. The un-mineralized fraction of nitrogen proved to be
383 present as oxamic acid, as also observed by [Pereira et al. \(2013\)](#).

384 **Insert Figure 3**

385 The OTC removal rate showed a 1.6-fold increase when the flow rate increased from 50
386 to 75 L h⁻¹. A further increase on flow rate from 75 to 100 L h⁻¹ resulted in an increment
387 on the reaction rate of only 1.2 times (see Table 3). This indicates that the
388 hydrodynamic conditions do not considerably change between 75 to 100 L h⁻¹. Under
389 this condition (100 L h⁻¹), an OTC removal >90% is reached after 5 min of reaction (0.4
390 kJ L⁻¹ of accumulated energy), with a photonic efficiency (ξ) (number of OTC
391 transformed molecules divided by the number of incident photons) of 13.7%.

392 Comparing the degradation of the OTC molecule in UPW matrix by the two reactors
393 under study in the best conditions found for each one (Fig. 4), it was practically similar
394 in both reactors. On the other hand, FluHelik reactor showed to be more efficient (1.3
395 times) in terms of mineralization (77%) when compared with the Jets reactor (61%), for

396 the same accumulated UVC energy ($14.4 \text{ kJ}_{\text{UV}} \text{ L}^{-1}$). This indicates that the limiting step
397 of the reaction is the by-products removal, which is improved by the unique fluid
398 dynamics and irradiation properties of FluHelik reactor.

399 **Insert Figure 4**

400 *3.3 Effect of urban wastewater (UWW) matrix*

401 OTC removal by the UVC/H₂O₂ photochemical system was also evaluated for an UWW
402 fortified with 20 mg OTC L^{-1} . Fig. 5a shows an increment on OTC removal rate for
403 higher H₂O₂ doses using the Jets photoreactor. In fact, a 29-fold increase on OTC
404 oxidation rate is observed for the UVC/H₂O₂ system ($[\text{H}_2\text{O}_2]_0 = 500 \text{ mg L}^{-1}$) when
405 compared to direct photolysis (Table 3). Likewise, it was found that the highest OTC
406 oxidation rate using the UWW and the FluHelik photoreactor was reached with the
407 highest oxidant concentration applied ($[\text{H}_2\text{O}_2]_0 = 500 \text{ mg L}^{-1}$) (Fig 5b, Table 3). An
408 increase in the flow rate value from $50 \text{ to } 100 \text{ L h}^{-1}$, achieved a 1.2-fold improvement in
409 the OTC reaction rate (Table 3), associated with an higher degree of mixing inside the
410 system. Under those conditions ($500 \text{ mg H}_2\text{O}_2 \text{ L}^{-1}$; 100 L h^{-1}), 90% of OTC removal
411 was achieved after 7.5 min of reaction and using $0.6 \text{ kJ}_{\text{UV}} \text{ L}^{-1}$ and a final mineralization
412 of 71% was attained after 180 min of reaction and $14.4 \text{ kJ}_{\text{UV}} \text{ L}^{-1}$. Paralleling the reaction
413 rates in both matrices, a decrease of about 1.7 times was perceived when in the presence
414 of the UWW, using the same initial H₂O₂ dosage, mainly due to inner filter and
415 hydroxyl radicals scavenging effects (Wols and Hofman-Caris, 2012). Therefore, a
416 higher amount of oxidant is required to overcome those effects to obtain similar OTC
417 removals. In fact, when using the FluHelik photoreactor for both matrices, at the best
418 conditions, similar photochemical space time yield (PSTY) were observed (0.50 and
419 $0.53 \text{ m}^3_{\text{water}} \text{ m}^{-3}_{\text{reactor}} \text{ day}^{-1} \text{ kW}^{-1}$ using the UPW and UWW, respectively). This shows
420 the ability of the FluHelik reactor design to overcome matrix effects due to its unique

421 characteristics. It should be noted that the high H₂O₂ concentrations used in these
422 experiments were due to the low photon flows provided by the available UVC lamps. If
423 higher photon flows are provided (able of overcoming the wastewater inner filter
424 effects), lower initial doses of oxidant would be required to achieve the same OTC
425 oxidation rates.

426 **Insert Figure 5**

427 The FluHelik photoreactor showed a better performance than the conventional Jets
428 reactor during the OTC oxidation when in the presence of the UWW matrix at the same
429 operating conditions (500 mg L⁻¹ of H₂O₂ and 100 L h⁻¹) (Fig. 6, Table 3). In this case,
430 the helical movement of the fluid around the radiation source allows a more
431 homogeneous UV radiation distribution, enhancing the reaction rate. In fact, the Jets
432 reactor presents a solution flow pattern parallel to the radiation source, and
433 consequently, the liquid streams at higher distance from the light source receive a less
434 UV dose. FluHelik's unique fluid hydrodynamics also provided a more efficient oxidant
435 homolytic cleavage, allowing further removal of the remaining by-products. In fact, a
436 1.4 times higher hydrogen peroxide consumption was observed when using the
437 FluHelik reactor (Fig. 6), reaching a mineralization of 71% instead of 56% for the Jets
438 reactor, using the same accumulated UVC energy (14.4 kJ_{UV} L⁻¹).

439 **Insert Figure 6**

440 The FluHelik photoreactor was also evaluated for the treatment of an UWW matrix
441 spiked with OTC and 10 additional CECs (described in Table 2) from the 12 established
442 by Swiss legislation ([Hochstrat et al., 2015](#)) at residual concentrations (60 µg L⁻¹) using
443 the UVC/H₂O₂ photochemical process. The Swiss legislation establishes 80% removal
444 for 5 out of 12 indicator compounds (11 pharmaceuticals and 1 biocide) ([Hochstrat et](#)
445 [al., 2015](#)). An OTC removal of more than 80% can be achieved using only 10 mg L⁻¹ of

446 H₂O₂ (Table 2) after 30 min. In order to comply with the Swiss legislation, an oxidant
447 amount of 250 mg L⁻¹ is required to achieve 80% removal of 5 compounds after 30 min.
448 However, using a H₂O₂ dose of 500 mg L⁻¹, after 30 min of reaction, a removal
449 efficiency of 80% is obtained for all the 11 CECs.

450 *3.4 CECs removal by an UVC/H₂O₂ photochemical system using FluHelik/Jets* 451 *photoreactors in single pass flow mode*

452 In order to estimate the efficiency in a real scale implementation, tests were performed
453 in single pass flow mode (one passage through the reactor) instead of recirculating the
454 solution between the reactor and the feed tank (multiple passage). In this way it is also
455 ensured that only the hydrodynamic effect of the reactors is evaluated, excluding the
456 additional mixture promoted by the recirculation. Fig 7a shows an improvement on the
457 OTC removal from an UWW matrix, at the steady state conditions (5 times the
458 residence time), by the UVC/H₂O₂ process (500 mg H₂O₂ L⁻¹; 6 W; 100 L h⁻¹), using the
459 FluHelik photoreactor (18% OTC removal) instead of the Jets reactor (15% OTC
460 removal). In fact, the longer residence time of FluHelik reactor (0.4 min) when
461 compared to that of the Jets reactor (0.3 min), along with the higher accumulated energy
462 (in a single passage) and with the lower dead volume zones due to the FluHelik helical
463 movement of the fluid contributed to the higher OTC removal.

464 Table 2 shows that much smaller removals of all the 11 CECs spiked in the UWW were
465 achieved by operating the FluHelik reactor in single passage mode (low residence time):
466 none of the compounds reach the removal imposed by Swiss legislation. The design of
467 the FluHelik photoreactor strongly favors the implementation of various reactors in
468 series, promoting its application in industry. Therefore, two FluHelik photoreactors
469 associated in series were tested for the removal of 20 mg L⁻¹ of OTC under the same
470 conditions previously tested with only one reactor. However, it was noticed that when

471 using the two reactors in series an OTC removal of only 31% was obtained after
472 reaching the steady state (Fig. 7b), a lower value than the one expected (36% - twice the
473 one achieved with only one reactor). In fact, when using 2 FluHelik reactors in series,
474 different velocity profiles can be found in the each reactor due to an increase in fluid
475 energy dissipation (pressure drop). Therefore, a new test was carried out doubling the
476 flow rate (200 L h^{-1}), corresponding to a residence time of 0.4 min. At these conditions,
477 an OTC removal of 36% was attained at steady state conditions. Therefore, when using
478 2 FluHelik reactors in series there is a minimum flow rate value to be used to achieve
479 fluid velocities profiles inside both photoreactors similar to when using only one
480 FluHelik reactor.

481 **Insert Figure 7**

482 Finally, a pilot-scale FluHelik reactor (95 W UVC lamp) under multiple pass flow mode
483 was also evaluated for the OTC removal using either UPW and UWW as solution
484 matrices. This system was operated at a flow rate of 7500 L h^{-1} , attaining a turbulent
485 regime inside the photoreactor ($\text{Re} = 15000$). Fig. 8a and 8b show that the highest OTC
486 oxidation rate was reached when using 500 mg L^{-1} of H_2O_2 for both reaction matrices.
487 However, a 1.5-fold decrease in the OTC kinetic rate (Table 3) was obtained for the
488 UWW when compared with UPW, as well as a slightly lower mineralization (52%
489 instead of 58%) using $4.6 \text{ kJ}_{\text{UV}} \text{ L}^{-1}$. It should be noted that, at the best conditions, when
490 using UPW, a higher photochemical space time yield (PSTY) at pre-pilot scale (0.85
491 $\text{m}^3_{\text{water}} \text{ m}^{-3}_{\text{reactor}} \text{ day}^{-1} \text{ kW}^{-1}$) when compared with the one at lab scale ($0.50 \text{ m}^3_{\text{water}}$
492 $\text{m}^{-3}_{\text{reactor}} \text{ day}^{-1} \text{ kW}^{-1}$) was noticed. This dissimilarity is mainly associated to the different
493 flow rates and UV fluence inside the reactors (distinct path length and UVC lamp
494 power). In addition, when using UWW, similar PSTY were observed at both scales
495 (0.57 and $0.53 \text{ m}^3_{\text{water}} \text{ m}^{-3}_{\text{reactor}} \text{ day}^{-1} \text{ kW}^{-1}$ at the pre-pilot scale and lab scale,

496 respectively). These data are in agreement with the results obtained by [Moreira et al.](#)
497 [\(2019\)](#) when comparing the degradation of a model compound, 3-amino-5-
498 methylisoxazole, using the FluHelik at the lab and pre-pilot scale; and indicate the
499 feasibility of scaling-up the FluHelik reactor.

500 **Insert Figure 8**

501 *3.5 Toxicity*

502 The percentage of embryo mortality at the end of the bioassays was similar among
503 treatments and remained at low levels, below 3% (data not shown). The total
504 abnormalities, the length and the yolk sac perimeter observed on zebrafish embryos
505 exposed to the initial and treated effluents are presented in Fig. 9. A significant increase
506 of total abnormalities (sum of tail abnormalities, lordosis anomalies and pericardial
507 oedema) was observed in embryos exposed to the UWW + 11 CECs, with 12.5% of
508 abnormal embryos in comparison with 1.4% of the control ($p < 0.05$). These
509 abnormalities were significantly reduced after the FluHelik photochemical treatment
510 (UWW + 11 CECs + Treatment), with values similar to the control (4.7% - $p > 0.05$).
511 The exposure to the UWW without the 11 CECs addition did not cause significant
512 abnormalities in the embryos ($p > 0.05$).

513 **Insert Figure 9**

514 It was also verified that the exposure to the UWW + 11 CECs significantly decreased
515 the length of the larvae and increase the yolk sac perimeter when compared with control
516 ($p < 0.05$). These endpoints return to control levels after the FluHelik photochemical
517 treatment (UWW + 11 CECs + Treatment). The yolk sac perimeter was also
518 significantly increased by the UWW without the 11 CECs.

519 When comparing the toxicity effects of the UWW + 11 CECs with those of the UWW +
520 11 CECs + Treatment (Fig. 9), it is evident that the UVC/H₂O₂ photochemical system

521 using the FluHelik photoreactor led to a significant decrease of the toxicity on zebrafish
522 embryos. In this sense, the treated wastewater had no significant effects on the total
523 abnormalities incidence, in the length of the larva and in the yolk sac perimeter. Thus, it
524 may be assumed that the degradation of pollutants present in the UWW fortified with 11
525 CECs by the UVC/H₂O₂ photochemical system with the FluHelik photoreactor did not
526 result in toxic transformation products to zebrafish embryos.

527 **4. Conclusions**

528 The FluHelik photoreactor showed to be an interesting system for UVC/H₂O₂
529 photochemical process applied to the removal of CECs from urban wastewaters, as a
530 polishing step, being able to comply with the Switzerland legislation and to
531 effectively reduce CECs toxicity to zebrafish embryos. Due to its unique configuration,
532 the FluHelik promotes an helical movement of the fluid around the irradiation source,
533 providing a more homogeneous UV radiation distribution (each fluid particle receives a
534 similar UVC radiation dosage), being able to overcome matrix effects in wastewaters
535 with low to moderate transmissibility (inner filter effects). Another advantage of this
536 reactor technology is its easy scalability, through its very simple and compact
537 arrangement in series, strongly promoting its use in industrial applications.

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636
637

638 **Figure Captions**

639 **Fig. 1.** FluHelik photoreactor scheme.

640

641 **Fig. 2.** Effect of H₂O₂ initial concentration and respective consumption (▼ - Direct
642 photolysis; ■ - 20 mg H₂O₂ L⁻¹; ● - 50 mg H₂O₂ L⁻¹; ▲ - 100 mg H₂O₂ L⁻¹; ◀ -
643 200 mg H₂O₂ L⁻¹; ▶ - 500 mg H₂O₂ L⁻¹; ◆ - 700 mg H₂O₂ L⁻¹) on the degradation of
644 OTC ([OTC]₀ = 20 mg L⁻¹) by a UVC/H₂O₂ process with (a) 4 W, (b) 6 W and (c) 11 W
645 UVC lamp; (d) OTC removal profiles at the optimized H₂O₂ concentrations for each
646 lamp (■ - 4W lamp and 100 mg H₂O₂ L⁻¹; ● - 6W lamp and 100 mg H₂O₂ L⁻¹; ▲ -
647 11W lamp and 500 mg H₂O₂ L⁻¹): Jets reactor; multiple pass flow mode; Q = 100 L h⁻¹,
648 pH 7.5 and 25 °C.

649

650 **Fig. 3.** Effect of H₂O₂ initial concentration and respective consumption (▼ - Direct
651 photolysis; ■ - 100 mg H₂O₂ L⁻¹; ● - 200 mg H₂O₂ L⁻¹;
652 ▲ - 300 mg H₂O₂ L⁻¹; ◀ - 400 mg H₂O₂ L⁻¹) on the degradation of OTC by a
653 UVC/H₂O₂ process using the FluHelik photoreactor: [OTC]₀ = 20 mg L⁻¹; Q = 100 L h⁻¹
654 ¹; multiple pass flow mode; 6 W UVC lamp, pH 7.5 and 25 °C.

655

656 **Fig. 4.** OTC removal from a ultrapure water matrix by a UVC/H₂O₂ process, as well as
657 H₂O₂ consumption and mineralization efficiencies (open symbols) achieved when using
658 the (■) Jets reactor with 100 mg L⁻¹ of H₂O₂ and (●) the FluHelik reactor with 300 mg
659 L⁻¹ of H₂O₂: [OTC]₀ = 20 mg L⁻¹, Q = 100 L h⁻¹; multiple pass flow mode; 6 W UVC
660 lamp; pH 7.5 and 25 °C.

661

662 **Fig. 5.** Effect of H₂O₂ initial concentration and respective consumption (▼ - Direct
663 photolysis; ■ - 100 mg H₂O₂ L⁻¹; ● - 300 mg H₂O₂ L⁻¹, ▲ - 500 mg H₂O₂ L⁻¹) on the
664 degradation of OTC in a real urban wastewater matrix using a UVC/H₂O₂ process with
665 (a) a Jets reactor and (b) a FluHelik reactor: [OTC]₀ = 20 mg L⁻¹; Q = 100 L h⁻¹;
666 multiple pass flow mode; 6 W UVC lamp; pH 7.5 and 25 °C.

667

668 **Fig. 6.** OTC removal from a real urban wastewater by a UVC/H₂O₂ process, as well as
669 H₂O₂ consumption and mineralization efficiencies (open symbols) achieved when using
670 (■) the Jets reactor and (●) the FluHelik reactor: [OTC]₀ = 20 mg L⁻¹; Q = 100 L h⁻¹;
671 multiple pass flow mode; 6 W UVC lamp; [H₂O₂] = 500 mg L⁻¹; pH 7.5 and 25 °C.

672

673 **Fig. 7.** OTC removal from a real urban wastewater by a UVC/H₂O₂ process using (a)
674 the Jets reactor (■) and the FluHelik reactor (●) at 100 L h⁻¹ and (b) 1 FluHelik reactor
675 at 100 L h⁻¹ (■), 2 FluHelik reactors in series at 100 L h⁻¹ (●), 2 FluHelik reactors in
676 series at 200 L h⁻¹ (▲): [OTC]₀ = 20 mg L⁻¹; single pass flow mode; 6 W UVC lamp;
677 [H₂O₂] = 500 mg L⁻¹; pH = 7.5 and 25°C.

678

679 **Fig. 8.** Effect of H₂O₂ initial concentration and respective consumption (■ - 100 mg
680 H₂O₂ L⁻¹; ● - 300 mg H₂O₂ L⁻¹; ▲ - 500 mg H₂O₂ L⁻¹; ▼ - 700 mg H₂O₂ L⁻¹) on the
681 degradation of OTC in (a) a ultrapure water matrix and (b) a real urban wastewater
682 matrix by a UVC/H₂O₂ process using a FluHelik reactor at pilot-scale: [OTC]₀ = 20 mg
683 L⁻¹; Q = 7500 L h⁻¹; multiple pass flow mode; 95 W UVC lamp; pH = 7.5 and 25°C.

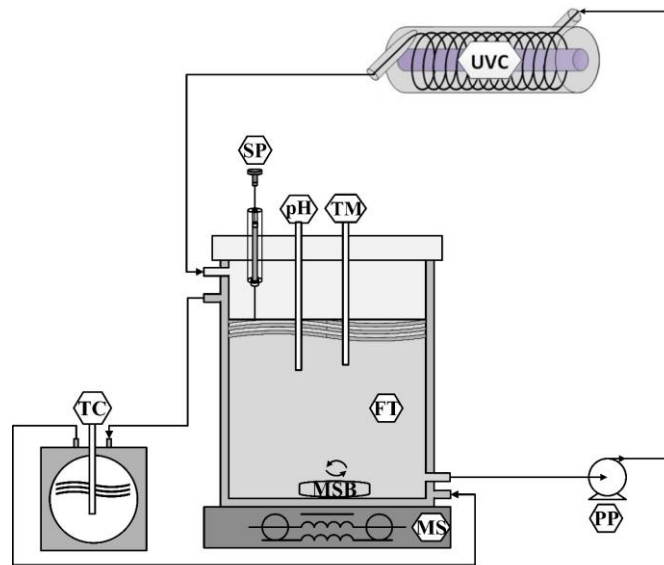
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685 **Fig. 9.** (a) Total abnormalities (%) and (b) larval length (■) and yolk sac (■) perimeter
686 (%) of *Danio rerio* embryos exposed to a real urban wastewater containing 11
687 pharmaceuticals before (UWW + 11 CECs) and after (UWW + 11 CECs + Treatment)
688 the UVC/H₂O₂ treatment in the FluHelik photoreactor plus the real urban wastewater
689 (UWW) without the 11 pharmaceuticals. Results are normalized to the respective
690 control assay; error bars indicate standard errors; bars with different letters or symbols
691 indicate significant differences ($p < 0.05$).

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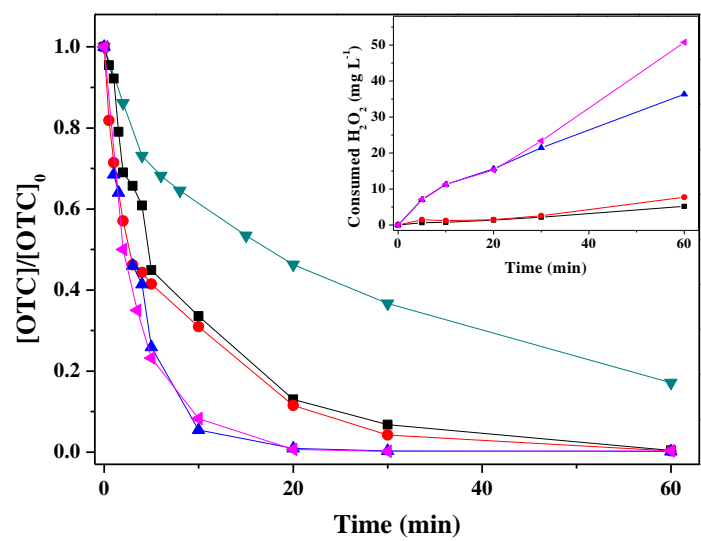
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694 **Figure 1**

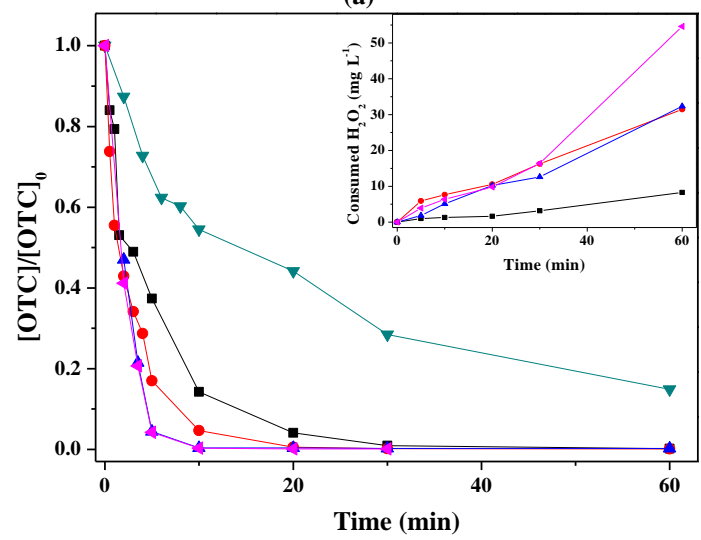


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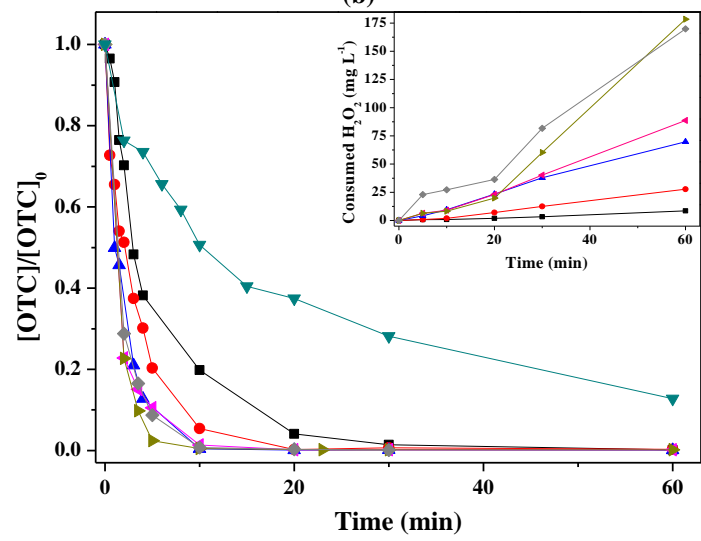
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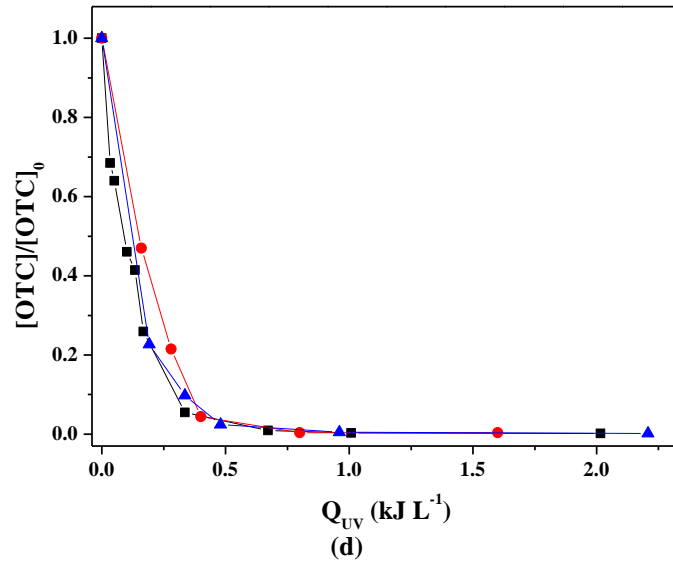
(a)



(b)



(c)

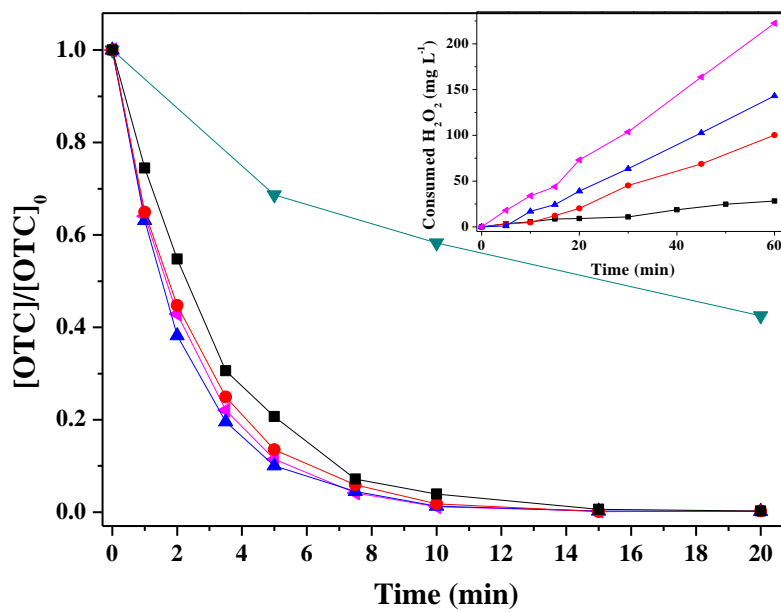


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700 **Figure 3**

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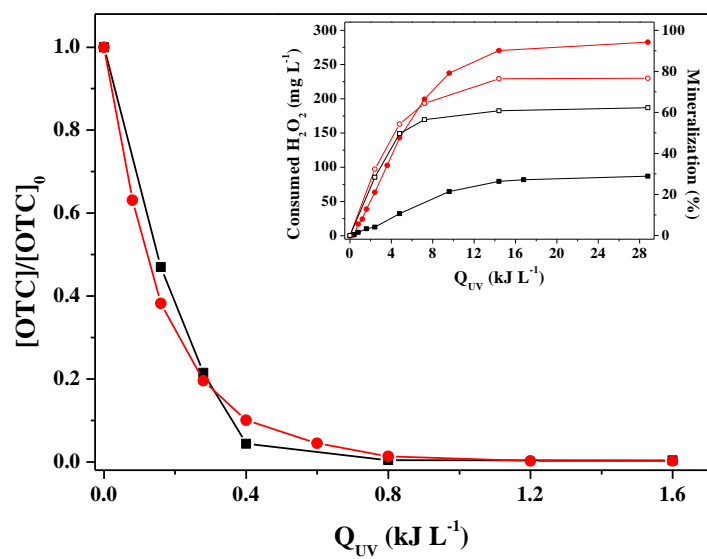
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707 **Figure 4**



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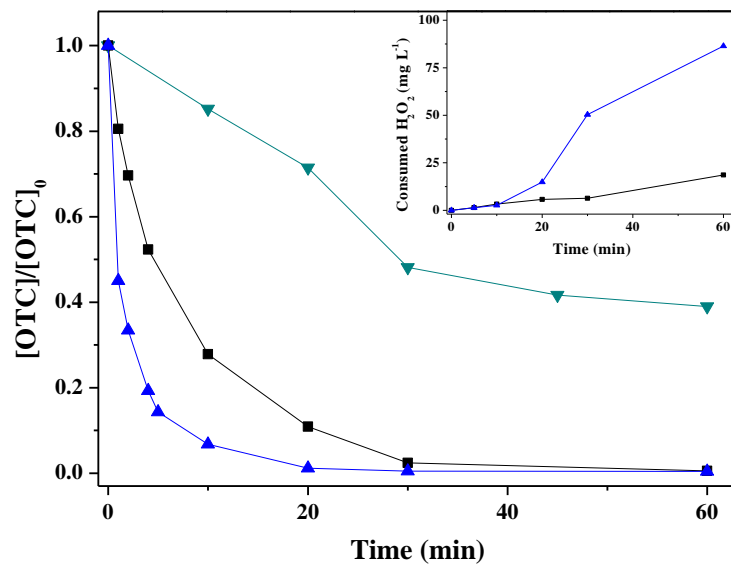
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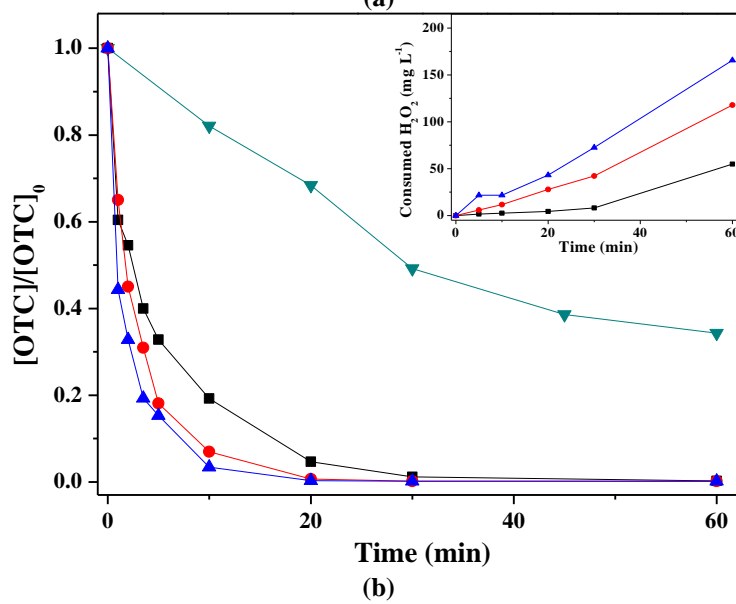
726 **Figure 5**

727



Time (min)

(a)



Time (min)

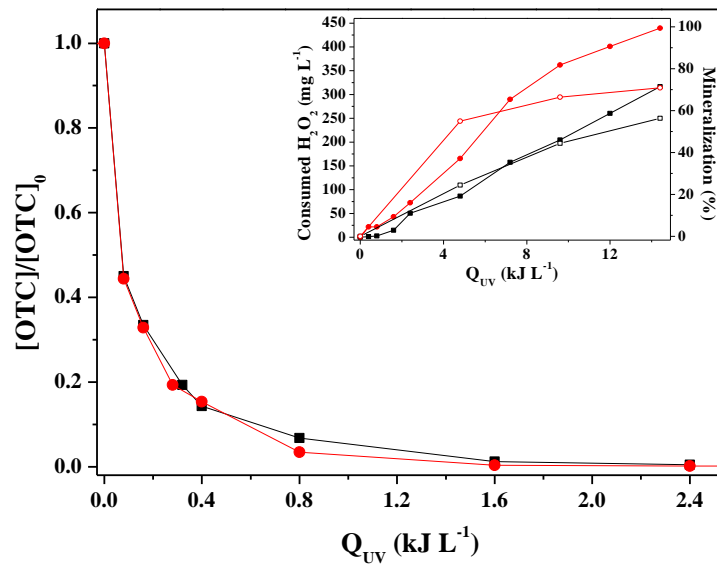
(b)

728

729

730 **Figure 6**

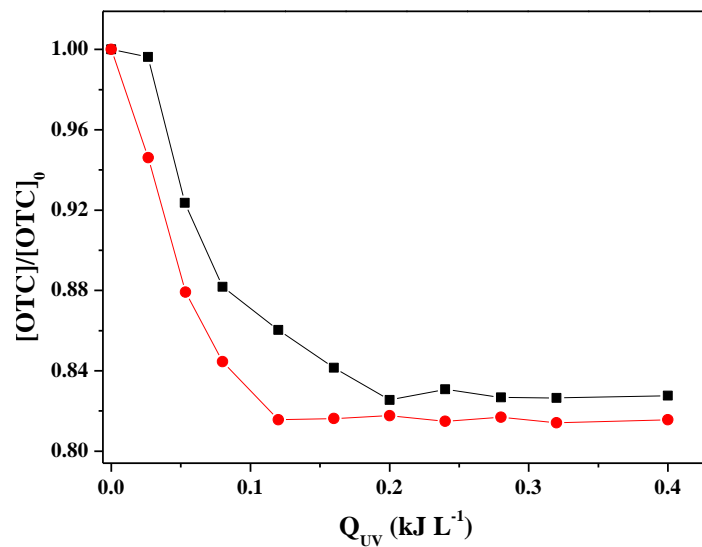
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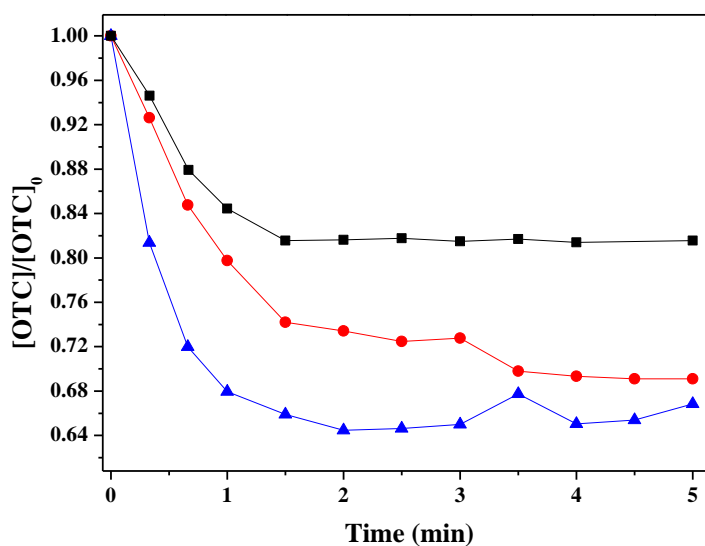
732

733 **Figure 7**

734



(a)



(b)

735

736

737

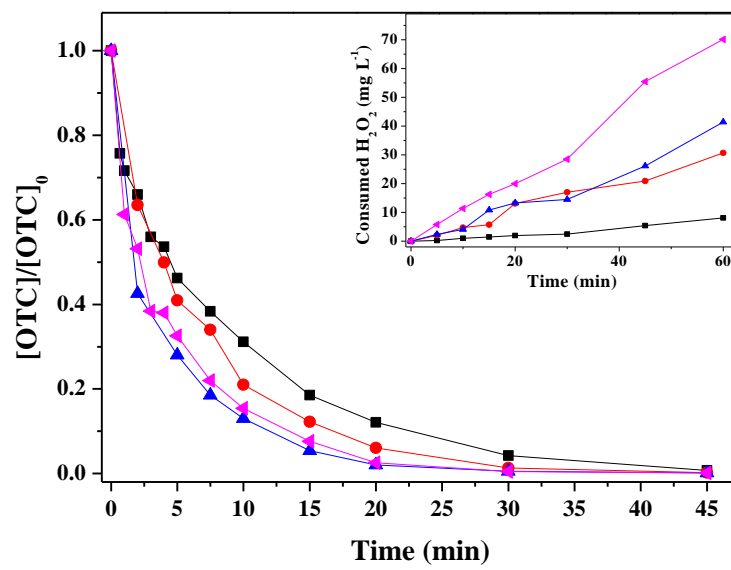
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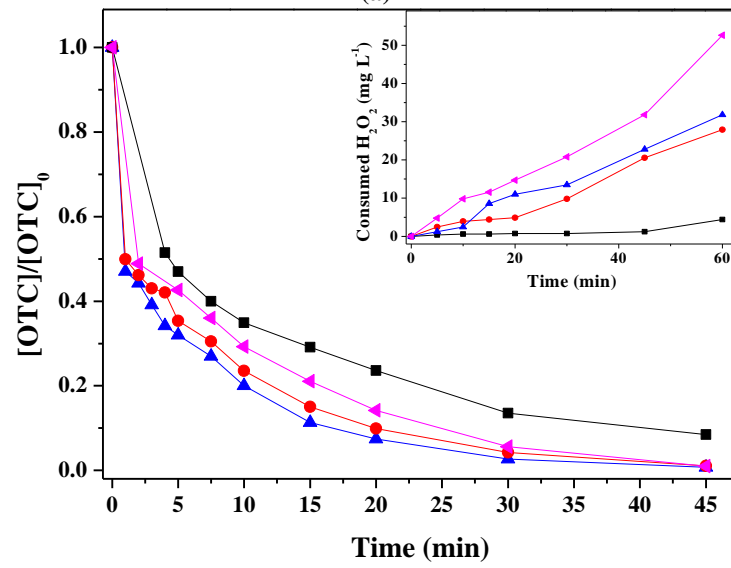
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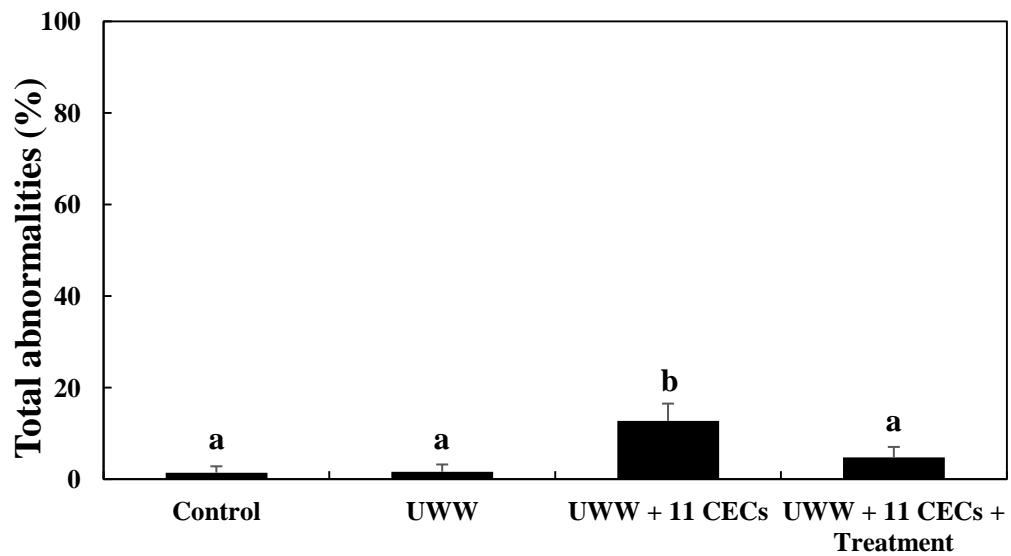
(a)



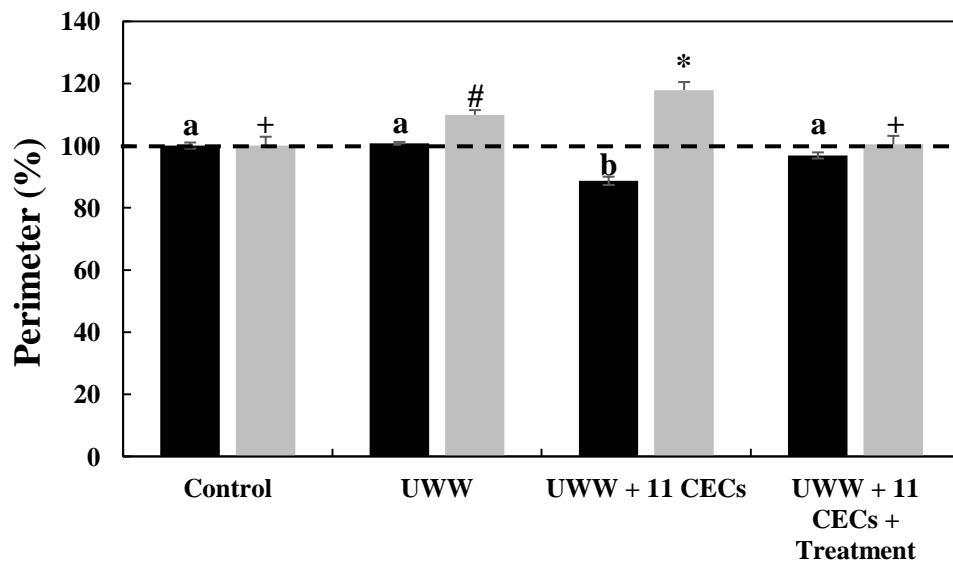
(b)

745 **Figure 9**

746



(a)



(b)

747

748 **Table 1.** Main physicochemical characteristics of the real urban wastewater collected
 749 after secondary treatment.

Parameter (units)	Values
Color	Pale/Yellow
Odor	n.d. ^a
pH	6.5
Temperature (°C)	24.7
Turbidity (UNT)	1.0
Conductivity ($\mu\text{S cm}^{-1}$)	883
Dissolved oxygen (mg L^{-1})	3.8
Redox potential (mV)	-10
Total dissolved carbon (mg L^{-1})	51
Dissolved inorganic carbon (mg L^{-1})	33
Dissolved organic carbon (mg L^{-1})	18
Chemical oxygen demand ($\text{mg O}_2 \text{L}^{-1}$)	56
Total dissolved iron (mg L^{-1})	0.26
Absorbance at 254 nm (AU)	0.21
Total suspended solids (mg L^{-1})	1.7
Volatile suspended solids (mg L^{-1})	1.7
Total dissolved nitrogen (mg L^{-1})	3.9
Total dissolved organic nitrogen (mg L^{-1})	2.7
Ammonium - N-NH_4^+ (mg L^{-1})	1.1
Nitrite - N-NO_2^- (mg L^{-1})	<0.02
Nitrate - N-NO_3^- (mg L^{-1})	0.09
Bromide - Br^- (mg L^{-1})	0.1
Chloride - Cl^- (mg L^{-1})	174
Phosphate - PO_4^{3-} (mg L^{-1})	12
Sulfate - SO_4^{2-} (mg L^{-1})	76
Calcium - Ca^{2+} (mg L^{-1})	57
Lithium - Li^+ (mg L^{-1})	<0.02
Magnesium - Mg^{2+} (mg L^{-1})	9.1
Potassium - K^+ (mg L^{-1})	29
Sodium Na^+ (mg L^{-1})	136
Total phosphorous - P (mg L^{-1})	4.8
Atenolol ($\mu\text{g L}^{-1}$)	1.1
Carbamazepine ($\mu\text{g L}^{-1}$)	4.8
Diclofenac ($\mu\text{g L}^{-1}$)	2.8
Metformin ($\mu\text{g L}^{-1}$)	2.1
Sulfamethoxazole ($\mu\text{g L}^{-1}$)	2.4
Trimethoprim ($\mu\text{g L}^{-1}$)	3.7

^an.d. - Not detected.

751 **Table 2.** Effect of H₂O₂ initial concentration in the removal of 11 pharmaceuticals spiked in a real urban wastewater by UVC/H₂O₂
 752 photochemical system using a FluHelik photoreactor in multiple or single pass flow mode.

Name	Chemical Formula	Company	% Removal of ΣCECs in multiple pass flow mode (after 30 min)					% Removal of ΣCECs in single pass flow mode
			[H ₂ O ₂] ₀					
			10 mg L ⁻¹	25 mg L ⁻¹	50 mg L ⁻¹	250 mg L ⁻¹	500 mg L ⁻¹	
Azytromicyn	C ₃₈ H ₇₂ N ₂ O ₁₂	TCI	9.3	19	23	> 95	> 95	24
Naproxen	C ₁₄ H ₁₄ O ₃	AlfaAesar	60	68	79	> 99	> 99	26
Atenolol	C ₁₄ H ₂₂ N ₂ O ₃	AlfaAesar	27	42	62	> 99	> 99	35
Metformin	NH ₂ C(=NH)NHC(=NH)N(CH ₃) ₂ .HCl	AlfaAesar	1.9	6.0	6.7	72	90	15
Bezafibrate	C ₁₉ H ₂₀ ClNO ₄	AlfaAesar	40	51	66	> 99	> 99	18
Ibuprofen	C ₁₃ H ₁₈ O ₂	AlfaAesar	30	38	63	> 99	> 99	35
Trimethoprim	C ₁₄ H ₁₈ N ₄ O ₃	AlfaAesar	19	29	43	> 99	> 99	17
Carbamazepin	C ₁₅ H ₁₂ N ₂ O	ACROS organics	25	30	45	> 99	> 99	19
Sulfamethoxazole	C ₁₀ H ₁₁ N ₃ O ₃ S	TCI	98	99	> 99	> 99	> 99	25
Oxytetracycline	C ₂₂ H ₂₄ N ₂ O ₉ .HCl	AppliChem Panreac	92	96	98	> 99	> 99	32
Diclofenac	C ₁₄ H ₁₀ ClN.NaO ₂	Sigma Aldrich	96	98	97	> 99	> 99	31

753

754 **Table 3.** Pseudo-first order kinetic constants along with the corresponding coefficient of determination (R^2) and residual variance (S^2_r), photonic
 755 efficiencies (ξ) and photochemical space time yields (PSTY) for degradation of 20 mg L⁻¹ of OTC at pH 7.5 and 25 °C.

Experiment	[OTC] ₀ (mg L ⁻¹)	[H ₂ O ₂] (mg L ⁻¹)	Q (L h ⁻¹)	pH	$k \times 10^1$ (min ⁻¹)	k (L kJ ⁻¹)	R^2	S^2_r (mg L ⁻¹) ²	$r_p \times 10^2$ (mg L ⁻¹ min ⁻¹)	$\xi \times 10^2$	PSTY (m ³ _{water} m ⁻³ _{reactor} day ⁻¹ kW ⁻¹)
Jets photoreactor - UVC lamp of 6 W											
1.1	5.1	0	100	4.5	0.17 ± 0.01	0.21 ± 0.01	0.989	0.02	0.018 ± 0.001	0.11	0.02
1.2	5.0	0	100	7.5 ^a	0.30 ± 0.02	0.37 ± 0.03	0.963	0.08	0.032 ± 0.002	0.19	0.03
1.3	5.0	0	100	7.5	1.1 ± 0.1	1.4 ± 0.1	0.976	0.07	0.12 ± 0.01	0.70	0.12
Jets photoreactor - UVC lamp of 4 W											
2.1	21.3	0	100	7.5	0.41 ± 0.04	1.2 ± 0.1	0.963	2.0	0.19 ± 0.02	2.6	0.10
2.2	20.9	20	100	7.5	1.3 ± 0.1	4.0 ± 0.3	0.976	1.3	0.6 ± 0.1	8.5	0.34
2.3	21.8	50	100	7.5	1.5 ± 0.2	4.6 ± 0.5	0.963	2.0	0.7 ± 0.1	10.3	0.40
2.4	19.9	100	100	7.5	2.7 ± 0.1	7.8 ± 0.4	0.988	0.6	1.2 ± 0.1	16.1	0.69
2.5	20.0	200	100	7.5	2.8 ± 0.1	8.2 ± 0.3	0.999	0.1	1.23 ± 0.04	17.2	0.73
Jets photoreactor - UVC lamp of 6 W											
3.1	20.9	0	100	7.5	0.52 ± 0.05	0.65 ± 0.06	0.961	2.0	0.24 ± 0.02	1.4	0.06
3.2	22.0	20	100	7.5	2.4 ± 0.3	3.1 ± 0.3	0.968	2.1	1.2 ± 0.1	6.9	0.26
3.3	22.9	50	100	7.5	4.0 ± 0.3	5.0 ± 0.4	0.972	1.6	2.0 ± 0.2	11.7	0.43
3.4	20.2	100	100	7.5	5.0 ± 0.2	6.3 ± 0.2	0.998	0.1	2.2 ± 0.1	13.0	0.54
3.5	21.0	200	100	7.5	4.7 ± 0.2	5.9 ± 0.3	0.996	0.3	2.1 ± 0.1	12.6	0.51
3.6	20.8	100	50	7.5	2.2 ± 0.1	2.8 ± 0.2	0.992	0.5	1.0 ± 0.1	5.9	0.24
3.7	21.4	100	75	7.5	3.0 ± 0.3	3.7 ± 0.4	0.982	0.8	1.4 ± 0.1	8.2	0.32
Jets photoreactor - UVC lamp of 11 W											
4.1	21.5	0	100	7.5	0.58 ± 0.04	0.61 ± 0.05	0.962	1.8	0.27 ± 0.02	1.3	0.05
4.2	21.0	20	100	7.5	2.0 ± 0.2	2.1 ± 0.2	0.983	1.2	0.9 ± 0.1	4.4	0.18
4.3	22.0	50	100	7.5	3.5 ± 0.2	3.6 ± 0.2	0.974	1.2	1.7 ± 0.1	8.2	0.32
4.4	21.3	100	100	7.5	5.18 ± 0.02	5.40 ± 0.02	0.999	0.1	2.40 ± 0.01	11.8	0.47
4.5	21.3	200	100	7.5	6.4 ± 0.1	7 ± 1	0.986	0.9	2.94 ± 0.03	14.4	0.57
4.6	20.6	500	100	7.5	7.2 ± 0.2	7.5 ± 0.2	0.999	0.1	3.2 ± 0.1	15.9	0.65
4.7	20.0	700	100	7.5	5.7 ± 0.3	5.9 ± 0.3	0.996	0.2	2.5 ± 0.1	12.1	0.51

FluHelik photoreactor - UVC lamp of 6 W											
5.1	22.1	0	100	7.5	0.5 ± 0.1	0.63 ± 0.06	0.983	1.21	0.24 ± 0.02	1.4	0.05
5.2	22.8	100	100	7.5	3.2 ± 0.1	4.0 ± 0.1	0.999	0.09	1.58 ± 0.03	9.3	0.35
5.3	22.2	200	100	7.5	4.0 ± 0.1	5.1 ± 0.1	0.999	0.04	1.95 ± 0.03	11.5	0.44
5.4	22.8	300	100	7.5	4.7 ± 0.1	5.8 ± 0.1	0.999	0.02	2.32 ± 0.03	13.7	0.50
5.5	22.6	400	100	7.5	4.32 ± 0.03	5.40 ± 0.04	0.999	0.01	2.12 ± 0.01	12.5	0.47
5.6	21.4	300	50	7.5	2.46 ± 0.03	3.08 ± 0.04	0.999	0.04	1.15 ± 0.02	6.8	0.27
5.7	21.3	300	75	7.5	4.0 ± 0.1	5.1 ± 0.1	0.999	0.06	1.87 ± 0.03	11.0	0.44
Real Urban Wastewater - Jets photoreactor - UVC lamp of 6 W											
6.1	19.8	0	100	7.5	0.16 ± 0.01	0.20 ± 0.01	0.996	0.19	0.07 ± 0.02	0.4	0.02
6.2	20.5	100	100	7.5	1.5 ± 0.1	1.9 ± 0.1	0.988	0.75	0.66 ± 0.05	3.9	0.16
6.3	20.9	500	100	7.5	4.6 ± 0.4	5.8 ± 0.5	0.986	0.78	2.1 ± 0.2	12.4	0.50
Real Urban Wastewater - FluHelik photoreactor - UVC lamp of 6 W											
7.1	20.3	0	100	7.5	0.2 ± 0.1	0.23 ± 0.01	0.976	0.97	0.08 ± 0.01	0.5	0.02
7.2	20.4	100	100	7.5	2.0 ± 0.1	2.5 ± 0.2	0.992	0.47	0.9 ± 0.1	5.1	0.21
7.3	20.1	300	100	7.5	3.3 ± 0.1	4.2 ± 0.1	0.998	0.09	1.45 ± 0.04	8.5	0.36
7.4	19.5	500	100	7.5	4.9 ± 0.4	6.1 ± 0.5	0.989	0.58	2.1 ± 0.2	12.1	0.53
7.5	19.9	500	50	7.5	4.0 ± 0.4	5.0 ± 0.4	0.981	0.98	1.7 ± 0.2	10.1	0.43
7.6	19.4	500	75	7.5	4.7 ± 0.4	5.8 ± 0.5	0.987	0.66	2.0 ± 0.2	11.6	0.50
Pilot-scale FluHelik photoreactor - UVC lamp of 95 W											
8.1	20.4	100	7500	7.5	1.3 ± 0.1	5.1 ± 0.3	0.983	0.69	0.58 ± 0.03	0.35	0.44
8.2	20.7	300	7500	7.5	1.6 ± 0.1	6.4 ± 0.4	0.982	0.78	0.74 ± 0.04	0.45	0.55
8.3	21.5	500	7500	7.5	2.5 ± 0.1	9.7 ± 0.1	0.999	0.01	1.18 ± 0.01	0.72	0.85
8.4	20.5	700	7500	7.5	2.3 ± 0.1	9 ± 1	0.980	0.80	1.0 ± 0.1	0.63	0.78
Real Urban Wastewater - Pilot-scale FluHelik photoreactor - UVC lamp of 95 W											
9.1	20.8	100	7500	7.5	0.8 ± 0.1	3.2 ± 0.2	0.978	0.93	0.37 ± 0.03	0.23	0.28
9.2	19.8	300	7500	7.5	1.5 ± 0.1	5.7 ± 0.3	0.995	0.22	0.63 ± 0.03	0.38	0.49
9.3	20.6	500	7500	7.5	1.7 ± 0.1	6.6 ± 0.4	0.983	0.66	0.8 ± 0.1	0.47	0.57
9.4	19.6	700	7500	7.5	1.3 ± 0.1	5.0 ± 0.4	0.974	0.98	0.55 ± 0.04	0.34	0.43

^a without pH control

SUPPLEMENTARY MATERIAL

An Innovative Photoreactor, FluHelik, To Promote UVC/H₂O₂ Photochemical Reactions: Tertiary Treatment of an Urban Wastewater

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OTC and LMWCA analytical determinations

OTC concentration was followed by HPLC using a VWR Hitachi ELITE LaChrom LC fitted with a Merck LiChrosorb[®] RP-18 (5 μ m) LiChroCART[®] 125-4 column at 25 °C and a diode array detector (DAD). The equipment was operated in gradient mode using as mobile phase a mixture of acetonitrile/methanol/0.014M oxalic acid with ratios of 10:10:80 (v/v) from 0 to 3 min, 15:10:75 (v/v) from 3 to 5 min, 20:10:70 (v/v) from 5 to 7 min, and 10:10:80 (v/v) from 7 to 14 min. The flow rate was 0.8 mL min⁻¹. Samples of 50 μ L were injected and the DAD was set at 354 nm. The retention time was 5.8 min and the limits of quantification and detection were 1.2 and 0.3 mg L⁻¹ of OTC, respectively.

LMWCA concentrations were determined by ion-exclusion HPLC using the VWR Hitachi ELITE LaChrom LC fitted with a Phenomenex RezexTM ROA-Organic Acid H⁺ (8%) 300 mm \times 7.8 mm column at room temperature (25 °C). The mobile phase was 0.0025 M H₂SO₄ at a flow rate of 0.5 mL min⁻¹. Samples of 10 μ L were injected and the DAD was set at 210 nm.

Table S1 - Instrumental LC-MS/MS parameters

Compound	Retention time (min)	Parent ion (m/z)	Fragment ion (m/z)	Cone voltage (V)	Collision energy (V)	ESI mode	LOQ ng mL⁻¹
Metformin	1.3	130	60	30	15	Pos.	0.04
		130	71	30	20		
Carbamazepine	8.3	237	194	20	25	Pos.	0.01
		237	179	20	40		
Sulfamethoxazole	6.4	254	92	30	25	Pos.	0.01
		254	156	30	15		
Atenolol	5.1	267	145	40	25	Pos.	0.02
		267	190	40	20		
Trimethoprim	6.2	291	123	40	30	Pos.	0.02
		291	230	40	30		
Oxytetracycline	6.7	461	426	40	20	Pos.	0.01
		461	443	40	10		
Azytromicyn	7.5	749.5	116	70	40	Pos.	1.8
		749.5	591	70	25		
Ibuprofen	9.4	205	161	20	5	Neg.	0.1
		205	159	20	5		
Naproxen	8.4	229	170	20	15	Neg.	0.04
		229	185	20	10		
Diclofenac	9.1	294	250	33	10	Neg.	0.1
		294	252	33	10		
Bezafibrate	8.5	360	274	33	15	Neg.	0.01
		360	154	33	30		

Ionization was performed in positive mode using the following parameters: 3.5 and 1.5 kV (capillary voltage in positive and negative modes, respectively), 150°C (source temperature), 350°C (desolvation temperature), 650 L/h (desolvation gas-N² flow) and 10 L/h (cone gas- N² flow). Collision energy (CE) and cone voltage (CV) values were adjusted individually for every transition (parent/fragment pair). For every compound the transitions selected for quantification are shown in the upper row and the qualifier transitions are shown in the lower row.

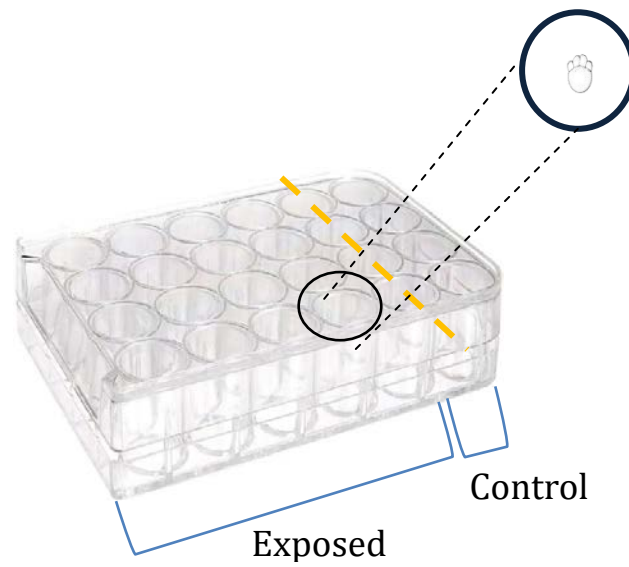


Figure S1 - Schematic representation of the experimental setup. In each 24-well plate was allocated one treatment (20 embryos; 1 embryo per well) plus an internal plate control (4 embryos). All the 24-well plates were maintained on a water bath at 26.5 ± 0.5 °C for 80 h.

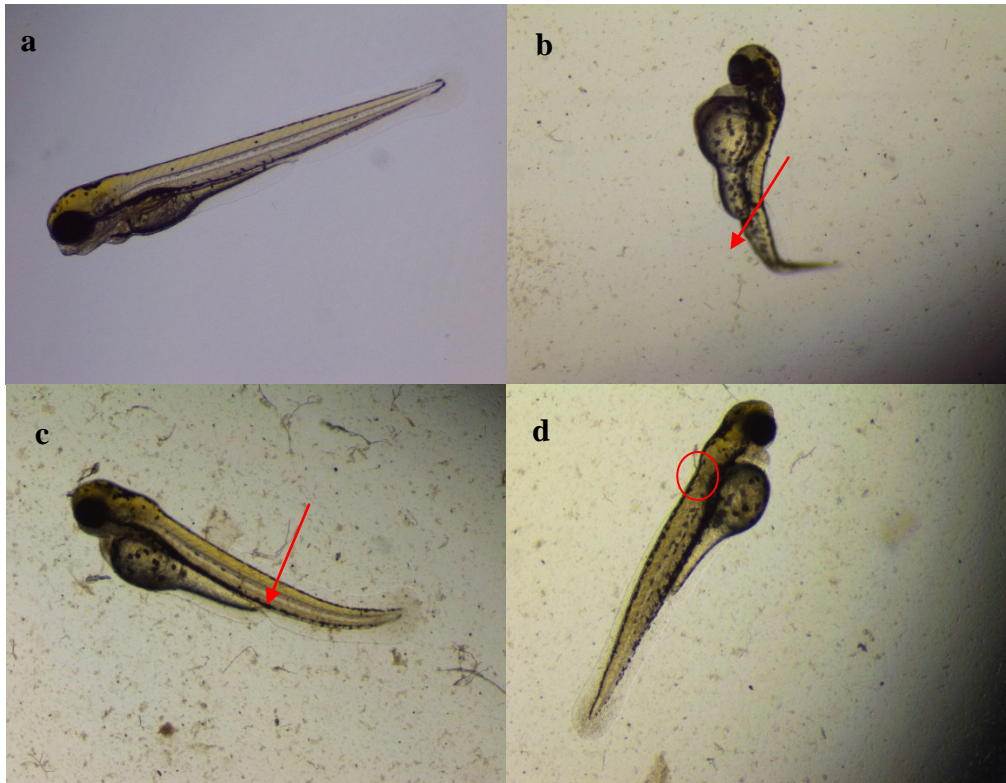


Figure S2 - Examples of different abnormalities detected in zebrafish embryos at 80 h: (a) normal embryo; (b) tail abnormality; (c) lordosis; (d) pericardial oedema.