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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1701128> since 2019-05-07T17:46:10Z

Published version:

DOI:10.1016/j.watres.2013.06.024

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(Article begins on next page)

The impact of the hydroxyl radical photochemical sources on the rivastigmine drug transformation in mimic and natural waters

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Abstract

In this paper we investigated the degradation of the rivastigmine drug induced by hydroxyl radical in synthetic and natural waters focusing on both reactivity and photoproduct identification. The hydroxyl radical formation rate was quantified by using terephthalic acid as trapping molecule and it was related with the rivastigmine degradation rate. The second order rate constant between hydroxyl radical and rivastigmine was estimated to be $\sim 5.8 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. Irradiation of rivastigmine in three natural waters (rain, lake and river) and comparison with degradation rates observed in synthetic solutions using nitrite, nitrate and hydrogen peroxide suggest that, in addition to hydroxyl radical, also nitroderived radicals (NO/NO_2) are responsible for the pollutant degradation in natural media. In fact, the evaluated degradation rates in three natural waters are greatly higher than those estimated considering only the reactivity with photogenerated hydroxyl radical. Using nitrites and nitrates as photochemical $\bullet\text{OH}$ source, the rivastigmine degradation cannot be described considering

28 only the hydroxyl radical reactivity suggesting that NO and NO₂ radicals could play a key
29 role during indirect degradation. Moreover main degradation products have been identified by
30 means of HPLC-MS. Hydroxylation of the aromatic ring as well as carbamate and amino
31 chain oxidation were suggested as main reaction mechanisms, but also nitroderived
32 compounds were characterized.

33 Finally polychromatic irradiations of three rivastigmine doped natural waters (rain, river and
34 lake) underlined the role of the indirect degradation that needs to be considered when direct
35 degradation of selected pollutants is negligible under environmental-like conditions.

36

37 **Keywords:** Photoinduced degradation; environmental fate; radical-induced transformation;
38 photooxidation; hydroxyl radical

39

40 **1. Introduction**

41 In recent years, the environmental pollution by emerging pollutants has become a major issue
42 and it is now well established that human pharmaceuticals effects might potentially play a
43 major role in other (non-mammalian) eco-organisms once reached the natural media (deep
44 and surface waters, soils ,...) (Khetan and Collins 2007). On the other hand, the impact and
45 fate of xenobiotics need to be investigated in order to asses the environmental potential risk.
46 Furthermore, the investigation of the environmental fate of different kinds of pharmaceuticals
47 is one of the main goals of recent European projects (Pharmas and Cytothreat). As stated in
48 the summary of the Pharmas project a classification system needs to be developed with the
49 intention of enabling all EU citizens to make their own informed decisions about the risk
50 posed by human pharmaceuticals to their health and to the health of the environment. The
51 results of this investigation will able EU regulators and policy makers to make better

52 informed decisions on the issue of pharmaceuticals in the environment (RIFERIMENTO_
53 link? <http://www.pharmas-eu.org/>).

54 In addition to priority pollutants many organic compounds used for human and veterinary
55 health, known as pharmaceuticals and personal care products (PPCPs), are regularly
56 introduced into the environment (McClellan and Halden 2010, Richardson and Ternes 2005,
57 Yu and Chu 2009). Many of these compounds, after application, are excreted by urine and
58 feces and are transported into sewage (Farré et al. 2008, Kümmerer et al. 1997). The sewage
59 treatment plants (STPs) are often unable to completely remove PPCPs that in this way enter
60 the environment, where they are frequently found (Andreozzi et al. 2003, Ellis 2006, Murray
61 et al. 2010). In the environment the pollutants may undergo biotic and/or abiotic
62 transformations (Cermola et al. 2007, Della Greca et al. 2008, DellaGreca et al. 2006, Jjemba
63 2006). In the case of surface waters, photochemical processes are the most important among
64 abiotic transformations. In such medium the indirect photolysis involving oxidant species
65 (singlet oxygen, hydroxyl radical, photoexcited organic matter) could be relevant for the
66 complete transformation of such compounds. The transformation products may be more
67 persistent and exhibit higher toxicity than parent compounds (DellaGreca et al. 2004). Drugs
68 are compounds made to have a biological activity and in the environment, even at small
69 concentration, they can interact with aquatic or non aquatic organisms with effects, often
70 unknown, on the ecosystem (Daughton and Ternes 1999, Halling-Sørensen et al. 1998,
71 Jørgensen and Halling-Sørensen 2000, Vaal et al. 1997). PPCPs include many classes of
72 heterogeneous compounds. More than 3000 pharmaceutical ingredients are registered
73 (Richardson et al. 2005, Ternes et al. 2004) and it is hard to predict the environmental fate and
74 the critical concentration for most of these compounds.

75 Many studies in the literature are about direct photodegradation of pollutants in organic
76 solvents and in water, identification of their photoproducts and the related formation

77 mechanisms (Burrows et al. 2002, de Bertrand and Barceló 1991, Doll and Frimmel 2003,
78 Sanz-Asensio et al. 1999). In order to predict the environmental fate of a pollutant it is
79 essential to study indirect photolysis as well, which is often the most important process. In
80 fact, in natural waters the pollutants may react with oxidant species more rapidly than with
81 direct light. Only few studies determine the values of second order kinetic constants with $\bullet\text{OH}$
82 (Charbouillot et al. 2012, Razavi et al. 2011), one of the most important oxidants in natural
83 waters.

84 Rivastigmine (Riv) is a drug used to treat patients with Alzheimer's disease and dementia
85 associated with Parkinson's disease (Mustazza et al. 2002). Rivastigmine hydrogen tartrate is
86 one of the few drugs approved in 2000 to treat Alzheimer's disease. This drug is a
87 cholinesterase inhibitor and acts on the central nervous system. One active site of the
88 molecule is the carbamate function (Mustazza et al. 2002, Yao and Li 2008), a function often
89 present in drugs and pesticides (insecticides and herbicides) (Gupta and Ramesh 2006).
90 Compounds with a carbamate function may undergo hydrolysis in the environment, but many
91 of these are found in ground and surface waters (Campbell et al. 2004, García de Llasera and
92 Bernal-González 2001).

93 Recently we investigated the direct degradation of rivastigmine under mono- and
94 polychromatic wavelengths underlining the phototransformation under environmental-like
95 conditions (Temussi et al. 2012).

96 In this work the transformation of rivastigmine toward hydroxyl radical generated using
97 different natural sources (H_2O_2 , NO_3^- , NO_2^-) was investigated showing the possible enhanced
98 transformation attributed to the $\text{NO}_2\bullet$ and $\text{NO}\bullet$ reactivity. Moreover three irradiations were
99 performed in natural waters (lake, river and rain) in order to compare the rivastigmine
100 degradation with previously obtained results in synthetic waters. A particular attention was

101 given to the degradation products showing the synergic effect of hydroxyl and NO_x radicals
102 on the rivastigmine transformation in waters.
103

104 **2. Materials and Methods**

105 **2.1 Chemicals**

106 Rivastigmine hydrogen tartrate (99 %) was purchased by Kemprotec. Hydrogen peroxide (30
107 %), sodium carbonate (99.5 %) and sodium nitrate (99 %) were purchased from Fluka;
108 sodium sulphate anhydrous (99.5 %) and sodium nitrite (98%) were purchased from Prolabo;
109 phosphoric acid (85% in water), sodium chloride (98 %), terephthalic acid (98 %) and 2-
110 hydroxyterephthalic acid (97 %) were purchased from Sigma Aldrich without additional
111 purification. All solvents were of HPLC grade and were purchased from Sigma-Aldrich.
112 Fresh solutions doped with nitrate, nitrite or H₂O₂ were prepared before each experiment. The
113 concentration of the stock solution of H₂O₂ in milli-Q water was determined using a molar
114 absorption coefficient of $38.1 \pm 1.4 \text{ M}^{-1} \text{ cm}^{-1}$ at 240 nm (Miller and Kester 2002).

115

116 **2.2 Irradiation experiments**

117 Aqueous solutions were irradiated in a thermostated cylindrical reactor. The 40 mL reactor,
118 cooled by water circulation at a temperature of $15 \pm 2 \text{ }^\circ\text{C}$ in order to limit thermal reactions,
119 was located at one focal point of the lamp in order to maintain a constant irradiation of the
120 whole sample and it was equipped on the top with a pyrex filter removing the wavelengths
121 lower than $\sim 285 \text{ nm}$. Samples were continuously stirred with a magnetic stirrer and a Teflon
122 bar to ensure homogeneity.

123 The emission spectrum of the Xenon lamp (see Figure 1) was recorded using a fiber optics
124 coupled with a CCD spectrophotometer (Ocean Optics USD 2000+UV-VIS). A reference
125 lamp (DH-2000-CAL, Ocean Optics) was used for calibration. The emission spectrum

126 reaching the reactor surface was calculated to be 4.31×10^{19} photons $\text{cm}^{-2} \text{s}^{-1}$ over the
127 wavelength range 290-400 nm.

128

129 **2.3 Detection of hydroxyl radicals**

130 The hydroxyl radical formation rate (R_{OH}^f) was determined by using terephthalic acid (TA) as
131 trapping molecule. TA reacts with hydroxyl radical leading to the formation of 2-
132 hydroxyterephthalic acid (TAOH) with a yield (Y_{TAOH}) determined as the ratio between the
133 initial formation rate of TAOH (R_{TAOH}^f) and the initial degradation rate of TA (R_{TA}^d). Y_{TAOH}
134 ranged between 12 and 30% depending on the pH and temperature of aqueous media
135 (Charbouillot et al. 2011). This method represents a simple and fast detection of
136 photogenerated $\cdot\text{OH}$ with a high sensitivity that allows detection of less the 10^{-18} M of
137 hydroxyl radical steady state concentration.

138 In order to calculate the formation rate of $\cdot\text{OH}$ as a function of different aqueous media
139 composition, different solutions were irradiated with the same setup used for rivastigmine
140 photochemical experiments. An aliquot of solution (3 mL) was withdrawn and put in a
141 fluorescence cuvette at fixed times. The cuvette was transferred into a Perkin-Elmer MPF 3 L
142 spectrofluorimeter and TAOH was quantified by using a calibration curve previously made
143 with standard solutions of TAOH. The concentration of TA used during all experiments was
144 included between 500 μM and 1 mM in order to trap all photogenerated $\cdot\text{OH}$.

145

146 **2.4 Natural waters**

147 Lake, river and rain waters, sampled on 2011, were collected in order to perform hydroxyl
148 radical measurement and rivastigmine degradation studies. River water and lake water come
149 from Artiere river and Chambon lake, respectively, both located in the Puy de Dôme region,
150 France. Artiere river passes through Clermont-Ferrand agglomeration, Chambon lake is in the

151 massif of Dore mountains at 877 m above sea level. Rain water was collected on the 1st
152 November 2011 at the "Cézeaux campus" (south of Clermont-Ferrand) located at 394 m of
153 altitude on the south of Clermont-Ferrand city.

154 Natural waters samples, collected using a glass bottle, were filtered on 0.20 µm membranes
155 (Minisart®, Sartorium Stedim) and stored under refrigeration. Irradiation experiments were
156 performed the day after, while, for analysis by ion chromatography (IC) inorganic and organic
157 anions concentrations were determined no more than 24 hours after sampling.

158

159 **2.5 Photoinduced degradation and data analysis.**

160 The experiments were performed either in Milli-Q water or in natural waters. The solutions in
161 Milli-Q water were prepared by mixing different concentrations of hydrogen peroxide (188
162 µM, 376 µM, 550 µM 752 µM), or nitrate (200 µM, 400 µM, 5 mM) or nitrite (5.4 µM, 10
163 µM, 50 µM, 100 µM) to a constant concentration of rivastigmine (45 µM). In order to analyze
164 the time evolution of rivastigmine an aliquot (200 µL) was taken each time, stored in the dark
165 and analysed by HPLC-UV. An HPLC system (Waters Alliance) equipped with a diode array
166 detector was used for rivastigmine analysis. An Eclipse XDB-C18 column (Agilent, 4.6 x 150
167 mm, 5 µm) and a gradient elution at a flow rate of 1.0 mL min⁻¹ were used. The gradient was:
168 at initial time 10 % acetonitrile and 90 % water acidified with 3‰ phosphoric acid, it was
169 kept for 7 min, and then followed by a linear gradient to 90 % acetonitrile within 8 min. Then,
170 the same ratio was maintained constant for 8 min followed by a linear gradient to 10 %
171 acetonitrile within 2 min. This ratio was maintained constant for 5 min. UV detector was set
172 at 210 nm. To prevent reactions after sampling, amber glass vials were used. Retention time
173 of rivastigmine was 11.2 min.

174 The time evolution of rivastigmine in the presence of photochemical sources of •OH could be
175 fitted with a pseudo-first order equation $C_0 = C_t \exp(-k t)$ where C_0 was the initial

176 rivastigmine concentration, C_t the concentration at time t and k the pseudo-first order
177 degradation rate constant. The error bars associated to the rate data represent 3σ , derived from
178 the scattering of the experimental data around the fitting curves (intra-series variability).

179 Ion chromatography analyses were performed using a DIONEX DX-320 instrument equipped
180 with an IonPac AG11 (guard-column 4×50 mm) and an IonPac AS11 (analytical column $5 \times$
181 250 mm) for anions. The elution step was performed using the following KOH concentration
182 gradient: at initial time $[\text{KOH}] = 0.2$ mM increasing up to 0.43 mM at 4.5 min, followed by a
183 linear gradient to 11.7 mM up to 18 min. The $[\text{KOH}]$ was then linearly increased up to 33.5
184 mM within 3 min. The flow rate was 1 mL min^{-1} .

185 Pure rivastigmine solution was obtained by dissolving the tartrate salt in a saturated Na_2CO_3
186 aqueous solution. The solution was then extracted using ethyl acetate (3×10 mL) and the
187 combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4 , before
188 filtration and evaporation under vacuum. Absence of tartrate was confirmed by NMR
189 spectroscopy and pure rivastigmine was stored in the dark at 5°C . Riv stability was checked
190 by comparison with UV-vis spectrum (recorded from 250 to 450 nm) of fresh extracted
191 solution (see Figure 1). pH values were measured with a Metrohm combined glass electrode,
192 connected to a Metrohm 713 pH-meter.

193 Hydrogen peroxide concentration was determined by spectrophotometric absorbance
194 detection of a solution in which *N,N*-diethyl-*p*-phenylenediamine (DPD) was involved in a
195 reaction catalysed by a peroxidase (POD). In the presence of POD, H_2O_2 oxidizes two
196 molecules of DPD to cationic radicals $\text{DPD}^{*\cdot}$ (Bader et al. 1988), which are stable and absorb
197 light at 551 nm ($\epsilon = 21\,000 \text{ M}^{-1} \text{ cm}^{-1}$). With a 10 cm optical path length cell, the detection
198 limit was $0.1 \mu\text{M}$ calculated as 3 times the standard deviation of field blanks. Each
199 measurement took less than 5 minutes.

200 Total organic carbon (TOC) and $\text{HCO}_3^-/\text{CO}_3^{2-}$ concentrations in natural waters were
201 quantified by TOC-5050A Analyzer (Shimadzu) using calibration curves previously
202 performed on standard solutions.

203

204 **2.6 Phototransformation products identification**

205 Degradation products identification was performed by means of an LC-MS system (Agilent
206 1100 Series, binary pump) equipped with an ESI ion source (MSD VL). The adopted column
207 was a Sphere Clone C18 column (Phenomenex, 4.6 x 250 mm, 5 μm) and the following
208 gradient elution was used: at initial time 30 % acetonitrile and 70 % water acidified with 1%
209 formic acid, followed by a linear gradient to 75 % acetonitrile within 55 min. Then, the same
210 ratio was maintained constant for 20 min. The flow rate was 0.4 mL min^{-1} and the UV
211 detector was settled at 254 nm.

212

213 **3. Results and discussion**

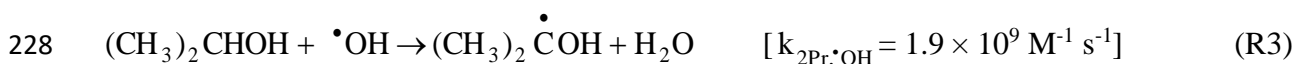
214 The goal of this work is to assess the reactivity of rivastigmine toward a wide range of
215 photochemical precursors of hydroxyl radical in the aquatic environment upon irradiation of
216 laboratory-made and natural solutions.

217

218 **3.1 Second order rate constant of rivastigmine with hydroxyl radical**

219 The second-order rate constant between Riv and hydroxyl radical was calculated by
220 competition kinetics with isopropanol ($(\text{CH}_3)_2\text{CHOH}$, 2Pr) added to the solution at different
221 concentrations. In Figure 2 we report the degradation rate of Riv obtained by irradiation of a
222 solution containing 45 μM of Riv, 380 μM of H_2O_2 and with addition of different isopropanol
223 concentrations ranging from 0 up to 1 mM. H_2O_2 was used as source of hydroxyl radical via
224 its photolysis (R1) under polychromatic irradiation.

225 The following reactions were included in the kinetic treatment of data reported in this work:



230 The experimental data were fitted by using the following equation (eq1).

231
$$R_{\text{Riv}}^d = \frac{R_{\cdot\text{OH}}^f k_{\text{Riv},\cdot\text{OH}} [\text{Riv}]}{k_{\text{Riv},\cdot\text{OH}} [\text{Riv}] + k_{\text{H}_2\text{O}_2,\cdot\text{OH}} [\text{H}_2\text{O}_2] + k_{2\text{Pr},\cdot\text{OH}} [2\text{Pr}]} \quad (\text{eq 1})$$

232 The data reported in Figure 2 were fitted with an equation of the type: $y = \frac{a}{1+bx}$ where a is

233 the ratio $\frac{R_{\cdot\text{OH}}^f k_{\text{Riv},\cdot\text{OH}} [\text{Riv}]}{k_{\text{Riv},\cdot\text{OH}} [\text{Riv}] + k_{\text{H}_2\text{O}_2,\cdot\text{OH}} [\text{H}_2\text{O}_2]}$, b is $\frac{k_{2\text{Pr},\cdot\text{OH}}}{k_{\text{Riv},\cdot\text{OH}} [\text{Riv}] + k_{\text{H}_2\text{O}_2,\cdot\text{OH}} [\text{H}_2\text{O}_2]}$ and x the

234 concentration of isopropanol. From the data fit we can obtain the second-order reaction rate

235 constant between hydroxyl radical and rivastigmine: $k_{\text{Riv},\cdot\text{OH}} = \frac{\frac{k_{2\text{Pr},\cdot\text{OH}}}{b} - k_{\text{H}_2\text{O}_2,\cdot\text{OH}} [\text{H}_2\text{O}_2]}{[\text{Riv}]}$

236 To the best of our knowledge, $k_{\text{Riv},\cdot\text{OH}}$ was determined for the first time to be $(5.8 \pm 0.3) \times$

237 $10^9 \text{ M}^{-1} \text{ s}^{-1}$. Additionally we obtained the formation rate of $\cdot\text{OH}$ from H_2O_2 photolysis under

238 the adopted irradiation condition: $R_{\cdot\text{OH}}^f = \frac{a (k_{\text{Riv},\cdot\text{OH}} [\text{Riv}] + k_{\text{H}_2\text{O}_2,\cdot\text{OH}} [\text{H}_2\text{O}_2])}{k_{\text{Riv},\cdot\text{OH}} [\text{Riv}]} = 9.6 \times 10^{-11}$

239 M s^{-1} in agreement with results obtained via direct quantification using TA.

240

241 **3.2 Reactivity toward different hydroxyl radical sources**

242 The stability of rivastigmine in aqueous solution was checked under dark conditions at pH 6.0
243 and no significant degradation was observed after 1 month, moreover direct photogredation
244 results to be negligible under our irradiation conditions. Rivastigmine (45 μM) was then
245 irradiated in the presence of different amounts of hydrogen peroxide, nitrate and nitrite. First
246 of all the hydroxyl radical formation rate (R_{OH}^{f}) was estimated as function of the
247 photochemical concentration by using 1 mM TA as reported in Table 1.

248 As expected, the degradation rate of rivastigmine ($R_{\text{Riv}}^{\text{d}}$) increases with the increasing of the
249 concentration of the photochemical source of $\bullet\text{OH}$. In Figure 3 we report the initial
250 degradation rate of rivastigmine $R_{\text{Riv}}^{\text{d}}$ as function of hydrogen peroxide and nitrite
251 concentration. In the case of hydrogen peroxide a competition due to the reaction R4 could be
252 predictable. Moreover using nitrite as $\bullet\text{OH}$ precursor the source reacts with photogenerated
253 hydroxyl radicals leading to the formation of nitrogen dioxide ($\text{NO}_2\bullet$) with a second order rate
254 constant $k_{\text{NO}_2\bullet, \bullet\text{OH}} = 1.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ (Mack and Bolton 1999). The plateau observed in figure
255 3 using 50 μM of nitrite could be explained taking into account its competitive role at high
256 concentrations toward photogenerated hydroxyl radical. In fact, considering the initial
257 concentration of Riv (45 μM) and nitrite (50 μM) in water we can argue that 34 % of
258 hydroxyl radical reacts with Riv while up to 66 % with nitrite. Additionally the percentage
259 significantly changes using 10 μM of nitrite. Under such conditions the 73 % of $\bullet\text{OH}$ reacts
260 with Riv while only 28 % with nitrite. Using nitrite and/or hydrogen peroxide a fraction of
261 photogenerated hydroxyl radical reacts also with their sources. From this assumption it is then
262 possible to quantify the fraction of hydroxyl radical reacting with rivastigmine for each
263 experimental point considering the initial concentrations of all species present in the solution
264 and related second order rate constants with $\bullet\text{OH}$. Therefore the correlation of the hydroxyl
265 radical formation rate (generated form hydrogen peroxide, nitrite and nitrate photolysis)

266 reacting with Riv??? is reported in Figure 4 showing that, under adopted concentrations, the
267 dependences are linear as expected. Nevertheless, using nitrite and nitrate, the rivastigmine
268 degradation increases compared to the value obtained in the presence of hydrogen peroxide.
269 Such trend could be explained considering the additional reactivity of NO_2^\bullet (and probably
270 NO^\bullet) generated via nitrate and nitrite direct photolysis or reaction with hydroxyl radical
271 leading to the formation of both hydroxylated and nitro-derived compounds (Vione et al.
272 2001, 2004). Such radicals could directly react with Riv and/or with radical intermediates
273 generated after H-abstraction driven by photogenerated hydroxyl radical. In any case
274 nitroderived radicals are generated and their reactivity toward Riv can explain the degradation
275 enhancement discussed in the following section.

276

277 **3.3 Product identification**

278 Three experiments were conducted using a rivastigmine concentration of 4 mM in the
279 presence of hydrogen peroxide 3.5 mM (experiment A), nitrates 15 mM (experiment B) and
280 nitrite 50 μM (experiment C). After 48 hours of irradiation the solutions were analyzed using
281 an HPLC-UV-MS.

282 Three main reaction-type mechanisms were discerned and the structures of the nineteen
283 identified products are proposed in Figure 5 on the basis of their mass spectra. The main
284 reaction, as expected in the presence $^\bullet\text{OH}$ precursors, is the hydroxyl radical addition to the
285 aromatic ring leading to the formation of product **A1**, while di- and tri- hydroxylated
286 compounds (**A2** and **A3**) are successively generated. Alcohol (**A4**), ketone (**A5**) and vinyl
287 (**A6**) derivatives could also be formed from direct photolysis via a homolytic and/or
288 heterolytic cleavage of the N-C bond as recently reported (Temussi et al. 2012) or via
289 hydroxyl radical attach as shown during mineralization of sulfamethoxazole (Wang et al.
290 2011).

291 Hydroxylation of **A4** and **A5** leads to the formation of **A7** and **A8** found only in trace levels
292 under the adopted irradiation conditions.

293 In the presence of nitrite and nitrate two species with $m/z [M+H]^+ = 280$ and 310 suggest that
294 nitration of the aromatic ring (**B1** and **B2**) could occur (Nélieu et al. 2004).

295 Few products were also found under all experimental conditions, with $m/z [M+H]^+ = 121$,
296 123 , 137 , 166 , 178 and 180 obtained from the carbamate chain cleavage or hydroxylation of
297 previously generated oxidation products. Moreover, high mass values were found ($m/z = 411$,
298 429 , 445 and 467) suggesting the formation of dimer molecules produced *via* radical
299 recombination.

300

301 **3.4 Irradiation in Natural waters**

302 Three natural waters (rain, lake and river) were doped with Riv ($45 \mu\text{M}$) in order to
303 investigate the role of hydroxyl radical sources on the rivastigmine degradation.

304 Inorganic anions, TOC and carbonates concentrations are reported in Table 2. The main
305 differences are the nitrate and nitrite concentrations found in river water: $231 \mu\text{M}$ and $\sim 7 \mu\text{M}$
306 respectively, while in rain and lake they are present only at μM levels or under the detection
307 limit. Also carbonates are more concentrated in river as well as chlorine and sulphate ions,
308 while similar TOC concentrations are found in the three samples.

309 Hydroxyl radical formation rate (R_{OH}^{f}) measured in lake and rain water using TA as trapping
310 probe (Charbouillot et al. 2011) are in agreement with previously reported values in literature
311 (Albinet et al. 2010, Vione et al. 2010). The calculated formation rate values range from $2.0 \times$
312 10^{-11} up to $6.5 \times 10^{-11} \text{ M s}^{-1}$ and from 3.2×10^{-12} to $4.0 \times 10^{-11} \text{ M s}^{-1}$ for rain and lake waters,
313 respectively. In our experiment the R_{OH}^{f} in river water was 6-7 times higher than in lake and
314 rain. Such value could be explained considering the high nitrate and nitrite concentration in
315 river samples. Moreover measured R_{OH}^{f} is well estimated showing that nitrite and nitrate

316 contributions represent about 57 and 22 % of the total hydroxyl radical formation in such
 317 medium. Nevertheless for rain and lake waters about 47 and 98 % of hydroxyl radicals are
 318 produced by other photochemical sources such as free iron, iron-complexes or chromophoric
 319 dissolved organic matter (CDOM) via its excited state (CDOM*). The possible impact of the
 320 carbonate radicals on the rivastigmine degradation rate founded in river water could be
 321 neglected. In fact carbonates can act as hydroxyl radical scavengers, generating carbonate
 322 radical, which could induce organic molecule degradation via electron transfer reaction and/or
 323 hydrogen abstraction with a quite low second order rate constant (10^{-6} - 10^{-7} $M^{-1} s^{-1}$) compared
 324 to $\bullet OH$. Concerning the "scavenging role", if we consider the relative low carbonates
 325 concentrations founded in natural waters used in this work (in river water we have 1 mM
 326 while for others the concentration not exceed 1.5×10^{-4} M) we can argue, taking into account
 327 the carbonate (HCO_3^-/CO_3^{2-}), nitrite and Riv initial concentrations and the second order rates
 328 constants with $\bullet OH$ ($k_{HCO_3^-, \bullet OH} = 8.5 \times 10^6 M^{-1} s^{-1}$ (Buxton et al. 1998) $k_{NO_2^-, \bullet OH} = 1.0 \times 10^{10}$
 329 $M^{-1} s^{-1}$ (Mack and Bolton 1999) and $k_{Riv, \bullet OH} = 5.8 \times 10^9 M^{-1} s^{-1}$ (this work)) that, in river
 330 water, about 0.25% of photogenerated hydroxyl radicals reacts with hydrogenocarbonate (at
 331 pH 7.9 more then 98% of total carbonate species are hydrogenocarbonate), ~22 % with nitrite
 332 and up then 78 % with rivastigmine.

333

334 **3.5 Radical driven degradation in natural selected waters**

335 In order to estimate the fate of the selected pollutant in natural waters, considering the double
 336 role of naturally occurring compounds, the hydroxyl radical steady-state concentration
 337 $[\bullet OH]_{ss}$ was measured in three natural waters by using TA (1 mM) as chemical probe
 338 following equation 2:

$$339 \quad -\frac{d[TA]}{dt} = k_{TA, \bullet OH} [TA][\bullet OH]_{ss} \quad (\text{eq 2})$$

340 where [TA] and $-d[TA]/dt$ are the initial concentration and the degradation rate of TA
 341 respectively, $k_{TA,\bullet OH}$ the second order rate constant between the probe and photogenerated
 342 hydroxyl radical ($3.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) (Charbouillot et al. 2011). The $[\bullet OH]_{ss}$ values range from
 343 $1.4 \times 10^{-18} \text{ M}$ for Lake up to $1.5 \times 10^{-17} \text{ M}$ for River water (Table 3). Experimental results are
 344 in agreement with those reported by Brezonik and Fulkerson-Brekken (Brezonik and
 345 Fulkerson-Brekken 1998) in 5 natural waters (river and lakes) for June mid-day sunlight
 346 radiation. The authors reported $[\bullet OH]_{ss}$ ranging from 2.5×10^{-17} up to $1.0 \times 10^{-15} \text{ M}$ and $9.0 \times$
 347 10^{-19} to $5.5 \times 10^{-17} \text{ M}$ in surface and whole water column respectively.

348 The hydroxyl radical steady-state concentration allows us the estimation of the Riv
 349 degradation rate considering only the hydroxyl radical reactivity and scavenging effect of
 350 naturally present compounds such as nitrite, carbonates ($\text{CO}_3^{2-}/\text{HCO}_3^-$) and CDOM using
 351 equation 3.

$$352 \quad R_{Riv}^{d,\bullet OH} = k_{Riv,\bullet OH} [Riv][\bullet OH]_{ss} - \sum_i k_{i,\bullet OH} [S_i][\bullet OH]_{ss} \quad (\text{eq 3})$$

353 Where $R_{Riv}^{d,\bullet OH}$ and [Riv] are the degradation rate of rivastigmine due to the $\bullet OH$ and its
 354 concentration, $\sum_i k_{i,\bullet OH} [S_i]$ is calculated as the sum of the contribution of the initial
 355 concentration of scavengers (nitrite, carbonates and CDOM) with the reaction rate constant
 356 with hydroxyl radical reported above. For these calculations the second order rate constant
 357 between CDOM and hydroxyl radical of $2.3 \times 10^4 \text{ mg of C L s}^{-1}$ (?ignoranza mia . che
 358 significa?) (Brezonik and Fulkerson-Brekken 1998) has been used. The R_{Riv}^d (M s^{-1})
 359 estimated considering only the role of hydroxyl radical using equation 3 (eq. 3) results to be
 360 less than those measured during irradiation in natural waters showing that additional
 361 degradation pathways are responsible for the rivastigmine degradation as observed under
 362 irradiation in synthetic waters.

363 Using the second order rate constant between hydroxyl radical and rivastigmine ($5.8 \times 10^9 \text{ M}^{-1}$
364 s^{-1}) founded in this work and highest $\bullet\text{OH}$ steady-state concentrations calculated in some
365 natural waters (10^{-15} - 10^{-16} M) (Brezonik and Fulkerson-Brekken 1998, Vione et al. 2010) the
366 $t_{1/2}$ of rivastigmine using equation 4

$$367 \quad t_{1/2} = \frac{\ln 2}{k_{\text{Riv}, \bullet\text{OH}} [\bullet\text{OH}]_{\text{ss}}} \quad (\text{eq 4})$$

368 could be estimated between 1 day up to 14 years?????. Nevertheless, as reported in the text
369 the real degradation is expected to be considerably enhanced due to the presence of
370 nitroderived reactive species.

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372 4. Conclusions

373 The degradation of rivastigmine drug was investigated toward different photochemical radical
374 sources found in natural waters. The first correlation of the formation rate of hydroxyl radical
375 underlines a participation of radicals such as $\text{NO}_x\bullet$ during polychromatic degradation in
376 mimic and natural media. The chemical structures of the main products generated *via*
377 oxidation of Riv have been suggested and hydroxylation, dimethylamino and carbamate
378 chains degradation as well as nitration have been identified as possible pathways to explain
379 the degradation products formation. Moreover the irradiation of Riv in three natural waters
380 underlines a key role of indirect degradation in natural media when direct photolysis is
381 negligible. The discrepancies between the Riv degradation rates calculated considering only
382 the effect of hydroxyl radical and those measured in three natural waters underline the
383 possible enhancement of the pollutant degradation in the presence of naturally occurring
384 compounds such as nitrate and nitrite. Such reactivity could sensibly accelerate the pollutant
385 degradation and need to be considered for a realistic half-life time estimation of organic
386 pollutants in natural media.

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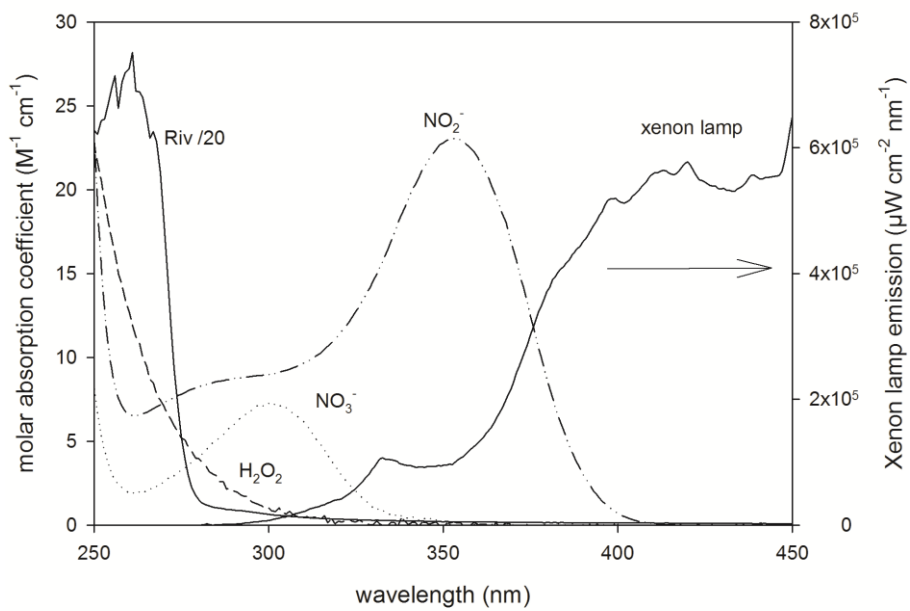
Figure Caption

- 1) Emission spectrum reaching the solution and molar absorption coefficients of the aqueous rivastigmine, nitrate, nitrite and H₂O₂ solutions.
- 2) Initial degradation rate of Riv under polychromatic irradiation of H₂O₂ 380 μM as a function of the isopropanol concentration. The solid curve shows the fit of experimental data with equation 1 and dashed lines denote the 95% confidence interval of this fit. The errors bars represent the 3σ based on the linear fit of experimental data.
- 3) Correlation between the initial degradation rate of Riv (R_{Riv}^d) with hydrogen peroxide and nitrite concentration under polychromatic irradiation. The errors represent the 3σ based on the linear fit of experimental data.
- 4) Correlation between the initial degradation rate of Riv (R_{Riv}^d) and the formation rate of hydroxyl radical ($R_{OH,Riv}^f$) reacting with rivastigmine. The photochemical sources

547 are: hydrogen peroxyde (empty circles) nitrites (full circles) and nitrates (empty
548 triangles). Dashed lines denote the 95% confidence of the linear fit.

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550 5) Proposed structures and formation mechanisms for the main rivastigmine
551 degradation products found in the presence of hydrogen peroxide, nitrites and
552 nitrates as photochemical sources of hydroxyl radical under polychromatic
553 irradiation.



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462 **Figure 1**

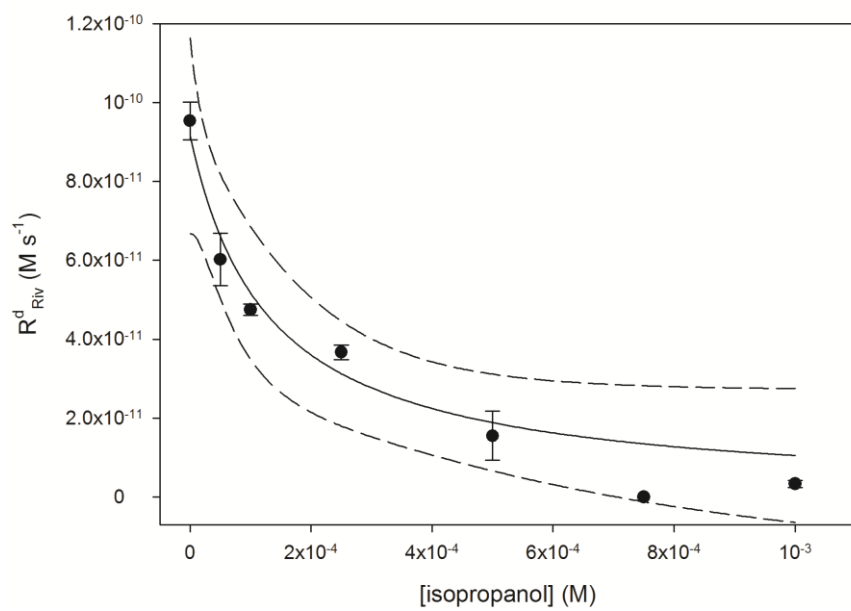
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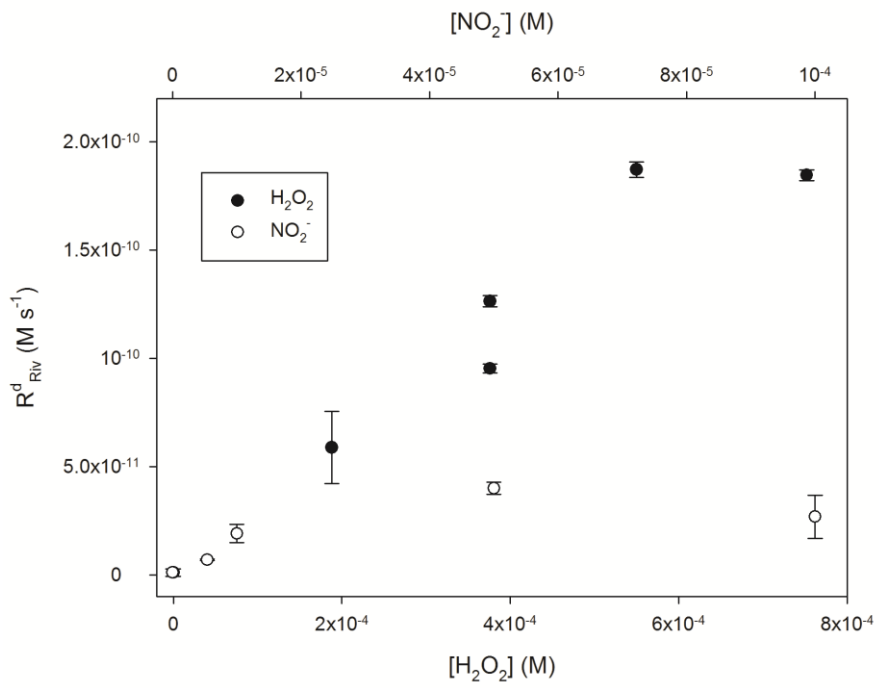
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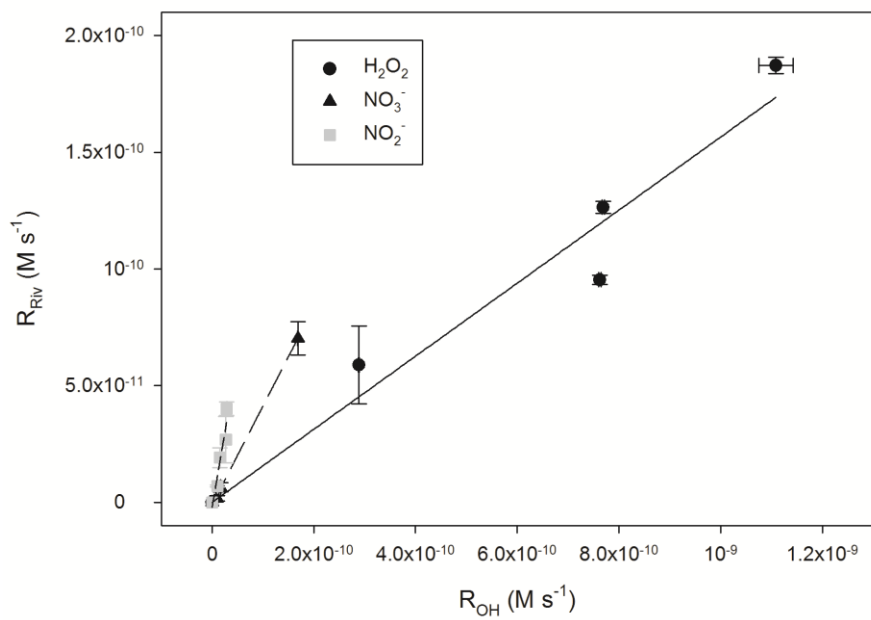
469 **Figure 2**

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472 **Figure 3**

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475 **Figure 4**

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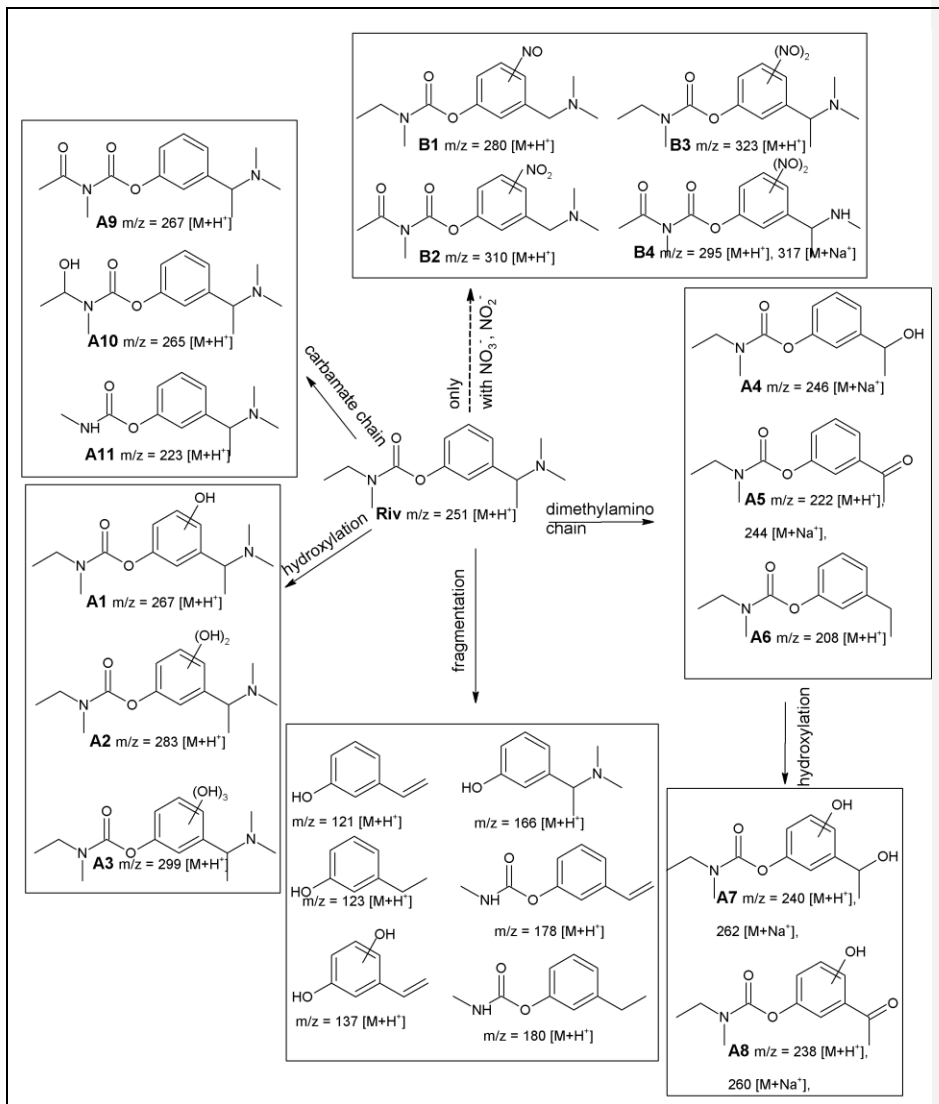
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Figure 5

	H ₂ O ₂	NO ₃ ⁻	NO ₂ ⁻
k (s ⁻¹)	(2.35 ± 0.13) x 10 ⁻⁶	(3.34 ± 0.21) x 10 ⁻⁸	(2.75 ± 0.06) x 10 ⁻⁶

Table 1: Rate constants of [•]OH formation from different sources (H₂O₂, NO₃⁻ or NO₂⁻).

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	Rain water	Lake water	River water
NO₃⁻ (M)	7.49 x 10 ⁻⁶	3.53 x 10 ⁻⁶	2.31 x 10 ⁻⁴
NO₂⁻ (M)	1.15 x 10 ⁻⁶	/	7.30 x 10 ⁻⁶
SO₄²⁻ (M)	3.98 x 10 ⁻⁶	5.09 x 10 ⁻⁵	3.75 x 10 ⁻⁴
Cl⁻ (M)	4.99 x 10 ⁻⁵	1.97 x 10 ⁻⁴	3.47 x 10 ⁻³
HCO₃⁻/CO₃²⁻ (M)	2.0	9.32	61.23
TOC (mg/L)	2.45	2.01	2.70
pH	7.2	6.6	7.9
R^f_{OH measured} (M s⁻¹)^a	(6.42 ± 0.53) x 10 ⁻¹²	(5.45 ± 0.08) x 10 ⁻¹²	(3.55 ± 0.02) x 10 ⁻¹¹
R^f_{OH predicted} (M s⁻¹)^b	3.42 x 10 ⁻¹²	1.21 x 10 ⁻¹³	2.80 x 10 ⁻¹¹
% [•]OH formation from NO₃⁻ ^c	4.0	2.2	22.4
% [•]OH formation from NO₂⁻ ^c	49.3	/	56.6
% [•]OH formation from other ^d	46.7	97.8	21.2
R^d_{Riv} (M s⁻¹)	(2.47 ± 1.20) x 10 ⁻¹²	(2.83 ± 1.63) x 10 ⁻¹²	(1.02 ± 0.05) x 10 ⁻¹¹

522 **Table 2:** Concentrations of nitrite, nitrate, HCO₃⁻/CO₃²⁻ and Total-organic carbon (TOC) of
523 natural waters used during rivastigmine experiments. ^a [•]OH formation rate obtained by using 1mM of
524 terephthalic acid as trapping molecule. ^b Theoretical [•]OH formation rate estimated by considering only
525 NO₂⁻ and NO₃⁻ as photochemical sources. ^c Contribution to the hydroxyl radicals attributed to NO₂⁻
526 and O₃. ^d Contribution to the hydroxyl radicals obtained by difference from known sources.

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