

1 **Removal of neonicotinoid insecticide clothianidin from water by ozone-based**
2 **oxidation: kinetics and transformation products**

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15
16 **ABSTRACT**

17
18 In this paper, reaction kinetics, degradation mechanisms and associated toxicity have
19 been assessed in detail concerning the abatement of neonicotinoid insecticide clothianidin
20 (CLO) by ozone-based oxidation in water. The second-order rate constants for the
21 reaction of CLO with molecular ozone (O₃) and hydroxyl radical (•OH) were determined
22 by the direct and competition kinetics methods, respectively, and estimated to be 103 M⁻¹
23 s⁻¹ and 3.7·10⁹ M⁻¹ s⁻¹. This suggested a (potential) higher contribution of the indirect
24 mechanism rather than the direct degradative pathway in the CLO ozonation process.
25 Additionally, CLO oxidation was studied through an O₃ and O₃/H₂O₂ system for three
26 complex real water matrices with distinct characteristics. DOC content was found to be
27 the main parameter responsible for making difficult the achievement of high CLO
28 degradations, whereas carbonate alkalinity did not exert a great impact on the process
29 efficiency. Results indicated that typical ozone doses (up to 1 mg O₃/mg DOC) were not
30 enough for the complete removal of this micropollutant, ranging only from 50 to 80%.
31 The study concerning the transformation products (TPs), performed by means of high-
32 resolution mass spectrometry, allowed to suggest potential degradation routes based on
33 the five major CLO-TPs identified. It was inferred that •OH was involved in the formation
34 of all TPs, whereas O₃ was only involved in the formation of two of them. A preliminary

35 toxicity assessment concerning CLO and its TPs during ozonation was conducted at three
36 different trophic levels via the ECOSAR software. Results showed that none of the
37 compounds was classified as very toxic for aquatic organisms, and all TPs exhibited
38 minor toxicity than the parent compound. Changes in molecular structure, such as
39 chlorothiazole ring breakdown, could be the main reason for this considerable decrease
40 in toxicity. Overall, the present study demonstrates that ozonation can be considered a
41 suitable technology alternative for CLO (and associated toxicity) abatement from aqueous
42 matrices.

43

44 **KEYWORDS**

45

46 Micropollutant, pesticide, transformation products, ozonation, hydroxyl radical

47

48 **1. Introduction**

49

50 Neonicotinoids are currently the most widely consumed class of insecticides worldwide
51 (Hirano et al., 2019). They act as selective agonists to nicotinic acetylcholine receptors
52 (nAChRs) in the central nervous system of insects (Goulson, 2013). Besides, they can
53 function successfully as systemic pesticides and thus be absorbed by crops (Pietrzak et
54 al., 2019). However, only around 5% of the active ingredient is taken up, and therefore
55 the residual neonicotinoid is released into the different environmental compartments
56 (Acero et al., 2019). Therefore, this class of insecticides can be frequently detected
57 worldwide in diverse surface and underground waters due mainly to their high-moderate
58 water solubility and low volatility (Klingelhöfer et al., 2022; Zhang & Lu, 2022). Several
59 research studies have highlighted the adverse effects of neonicotinoids on many non-
60 target organisms, such as pollinators (i.e., bees) or aquatic invertebrates (Sadaria et al.,
61 2016). In addition, recent studies suggest negative impacts of neonicotinoids on mammals
62 and human health, such as congenital heart defects (Li et al., 2022), autism spectrum
63 disorders (Yu et al., 2021), neurotoxicity and immunotoxicity, as well as reproductive
64 system impairments (Ponce-Vejar et al., 2022).

65

66 (*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine, better known as
67 clothianidin (CLO) is a second-generation neonicotinoid insecticide (Zhang et al., 2020).
68 Since its introduction in 2002, clothianidin has become one of the most widely applied

69 insecticides worldwide (You et al., 2022), accounting in 2012 for almost 15% of global
70 neonicotinoid sales (Bandeira et al., 2021). Its rapidly growing popularity was mainly
71 based on the outstanding properties initially exhibited by this compound, including a
72 broad insecticidal spectrum, high activity at low doses, long-term control effect,
73 distinguished systemic action, extensive application method and high crop safety
74 (Uneme, 2011). However, its global overuse during the last decades resulted in an
75 accumulation in the environment (Guo et al., 2021), thus considerably modifying the
76 ecological balance and posing a substantial threat to several non-target species (Y.-J. Lee
77 et al., 2022). In 2018, given the adverse effects that clothianidin poses to pollinators, more
78 specifically to honeybees, the European Union decided to ban its use on open field crops
79 and only authorize it in controlled permanent greenhouses (European Commission, 2018).
80 Despite this restriction, clothianidin has been frequently detected in various aquatic
81 resources (Yang et al., 2022) and even in agricultural regions of the American and Asian
82 continents (Alarcan et al., 2020) at relatively high levels (ng L^{-1} to $\mu\text{g L}^{-1}$) (Robinson et
83 al., 2021). Since its presence poses a serious threat to environmental, wildlife and human
84 safety, removing it from the different water compartments is of utmost importance.

85

86 Advanced Oxidation Processes (AOPs) are a group of technologies which have been
87 demonstrated to be highly effective in the removal of organic pollutants from different
88 water matrices (Fenoll et al., 2019), including pesticides in wastewater (Milojević-Rakić
89 et al., 2022), such as organophosphorus (Badawy et al., 2006) and neonicotinoid
90 pesticides (Cruz-Alcalde et al., 2017a; Fasnabi et al., 2019). Among them, ozone (O_3) is
91 one of the most widely used AOPs in full-scale water and wastewater treatment
92 applications (Umar et al., 2013), mainly due to its strong oxidizing power and capacity as
93 a disinfectant (González et al., 2020). This oxidation technique is based on reactions
94 between the target micropollutant and both molecular ozone and hydroxyl radical ($\bullet\text{OH}$)
95 (Y. Lee & von Gunten, 2016), resulting from O_3 decomposition during the process (von
96 Gunten, 2003). Ozone-based processes can further enhance the oxidation efficiency of
97 the conventional ozonation system since combining O_3 with other agents, such as
98 hydrogen peroxide (H_2O_2) can promote the generation of further hydroxyl radicals
99 (Ponnusamy et al., 2019; Wang et al., 2020). Despite having a lower concentration than
100 O_3 in aqueous solutions, $\bullet\text{OH}$ is generally more reactive to any molecule than ozone.
101 Hence, both species are expected to play an essential role in the degradation of target
102 contaminants (Du et al., 2019). However, and despite the fact that both species could

103 effectively remove unwanted chemicals from water and wastewater, the transformation
104 products (TPs) formed during the ozonation process may pose additional risks due to their
105 toxic character. Therefore, it is necessary to understand thoroughly this process when
106 applied to the specific removal of particular chemicals by assessing the reaction kinetics,
107 the transformation products, and the residual toxicity of the remaining species in the
108 treated water.

109

110 The present work aimed to delve deeply into the basics of the CLO ozonation process,
111 including the reaction kinetics, transformation products and related toxicity progression.
112 Therefore, the first step of this research was the determination of the rates of reaction
113 between this neonicotinoid and both ozone and hydroxyl radical. The potential removal
114 in full-scale applications was also studied through laboratory tests in real wastewater
115 samples. Furthermore, potential reaction routes and toxicity evolution were elucidated
116 through the investigation of the main CLO TPs generated.

117

118 **2. Materials and methods**

119

120 *2.1. Chemicals and reagents*

121

122 Clothianidin, *p*-chlorobenzoic acid (*p*CBA) and atrazine (ATZ) analytical standards, as
123 well as potassium indigotrisulfonate and *tert*-butanol (tBuOH, $\geq 99.7\%$), were purchased
124 from Sigma-Aldrich (Germany). Hydrogen peroxide (30% w/v), sodium hydrogen sulfite
125 (40% w/v), *tert*-Butanol, sodium hydrogen phosphate, sodium dihydrogen phosphate,
126 phosphoric acid, sodium hydrogen carbonate, sodium hydroxide and acetonitrile
127 analytical grade reagents were acquired from Panreac (Spain). Milli-Q water was
128 produced by a filtration system (Millipore, USA). Pure oxygen ($\geq 99.999\%$) was supplied
129 by Abelló Linde (Spain).

130

131 *2.2. Setup for oxidation experiments*

132

133 Oxidation experiments employing ozone were performed by mixing varying volumes of
134 aqueous O₃ stock solutions with solutions containing the target compound. Ozone stock
135 solutions (~ 1 mM) were prepared by continuously bubbling a small volume of Milli-Q
136 water with a gaseous oxygen stream containing ozone (~ 100 mg L⁻¹) for 30 min. Ozone

137 was generated using a 301.19 Labor Ozonator (Sander, Germany). All the ozonation
138 experiments were performed at a temperature of 20 ± 2 °C under controlled pH conditions.
139 The pH values were different depending on the experiment, although in any case achieved
140 using the addition of varying proportions of different phosphate salts (*i.e.*, Na_2HPO_4 ,
141 NaH_2PO_4 and H_3PO_4) to reach a total buffer concentration of 1 mM.

142

143 UV and UV/ H_2O_2 experiments were performed in a 2-L batch reactor equipped with a
144 4W, low-pressure Hg lamp supplied by Phillips (The Netherlands). The lamp was located
145 inside a quartz sleeve and placed symmetrically into the reaction vessel. The fluence rate
146 at 254 nm was determined by chemical actinometry using a 5 μM atrazine solution as an
147 actinometer (Canonica et al., 2008). The resulting value was $2.01 \cdot 10^{-5}$ Einstein $\text{m}^{-2} \text{s}^{-1}$.
148 All the photochemical experiments were performed at 20 ± 2 °C and pH 7. These
149 conditions were achieved using a cooling bath and a phosphate buffer (1 mM),
150 respectively. Homogeneous conditions were ensured using constant magnetic stirring.

151

152 2.3. Kinetic study and abatement during wastewater ozonation

153

154 Kinetic experiments to determine the second-order rate constant between O_3 and CLO
155 were performed by the addition of 10-15 mL aliquots of ozone stock solutions (~ 1 mM)
156 to aqueous solutions containing CLO (total volume: 250 mL), resulting in excess of the
157 oxidant. A closed glass bottle with minimal headspace was used as a reactor. The medium
158 pH was adjusted to a value of 7 and tBuOH was used as $\bullet\text{OH}$ scavenger. CLO and ozone
159 stock solutions were mixed to reach an initial concentration of 4 and 50 μM , respectively.
160 The medium was stirred for 10 s to correctly homogenize the resulting solution, then the
161 stirrer was stopped, and the sampling started. For 5 min, and at regular time intervals of
162 30 s, 1 mL aliquots were withdrawn from the reaction bottle and then split into two equal
163 volumes: 0.5 mL were quenched with 2.5 mL of an Indigo solution for dissolved ozone
164 measurements (Bader & Hoigné, 1981), while the other 0.5 mL were quenched with 10
165 μL of a 40% w/v sodium bisulfite solution before CLO quantification. These experiments
166 were performed in triplicate.

167

168 The second-order rate constant of CLO reaction with hydroxyl radical was determined by
169 competition kinetics with *p*CBA as the reference compound. The photolysis of H_2O_2 was
170 employed as the $\bullet\text{OH}$ source. Aqueous solutions containing both target and reference

171 compounds (1 μM both) were prepared and placed inside the photoreactor. The initial
172 concentration of hydrogen peroxide was 0.28 mM. Irradiation experiments were
173 conducted for 10 min, taking samples at 0, 0.5, 1, 1.5, 2, 3, 5 and 10 min. 0.5 mL aliquots
174 of these samples were employed for CLO and *p*CBA residuals determination. Blank
175 experiments without the addition of H_2O_2 were also carried out by following similar
176 procedures. All experiments were performed in triplicate.

177

178 Additional ozonation experiments were conducted to investigate the degradation of CLO
179 in actual wastewater effluents. To do that, 1.5-5 mL aliquots of the ozone stock solution
180 (~ 1 mM) were added to glass vials containing actual wastewater solutions (total volume:
181 25 mL) spiked with $100 \mu\text{g L}^{-1}$ of CLO, with the goal of testing ozone doses frequently
182 employed in full-scale treatments (3, 5 and 10 mg L^{-1}). The combination of hydrogen
183 peroxide with ozone was also tested, employing a typical $\text{H}_2\text{O}_2/\text{O}_3$ ratio of 0.5. In this
184 case, H_2O_2 was added to the reaction solution immediately before ozone addition. In all
185 cases, complete consumption of ozone was allowed before sample withdrawal for residual
186 CLO measurements. The main physicochemical parameters of the wastewater effluent
187 were pH, 7.8; DOC, 6.7 mg C L^{-1} ; Alkalinity, $233.2 \text{ mg CaCO}_3 \text{ L}^{-1}$; NO_2^- , 0.12 mg N L^{-1} .
188 To explore the effect of different water quality on CLO removal, oxidation tests were
189 performed with this effluent and with 1:1 dilution of this medium with ultrapure water,
190 the latter with and without compensation of alkalinity to the initial level via sodium
191 bicarbonate addition.

192

193 The residual concentration of CLO and *p*CBA were determined using a 1260 Infinity
194 High Performance Liquid Chromatograph (HPLC) equipped with a diode array detector
195 (DAD), all supplied by Agilent (USA). A Mediterranea Sea C18 column (250x4.6 mm
196 and particle size of $5 \mu\text{m}$) supplied by Teknokroma (Spain) was used. The mobile phase
197 consisted of a 1:1 volumetric mixture of acetonitrile and Milli-Q water acidified (pH 3)
198 with H_3PO_4 . The injection volume was set at $100 \mu\text{L}$, and the flow rate was kept at 1 mL
199 min^{-1} . Detection was carried out at 268 and 236 nm for CLO and *p*CBA, respectively.

200

201 2.4. *Evaluation of transformation products and associated toxicity*

202

203 In order to investigate CLO transformation products during ozonation and make possible
204 the elucidation of oxidation pathways, two extra sets of experiments were conducted. To

205 favour the formation of TPs by direct O₃ oxidation, one set of CLO ozonation experiments
206 was carried out under pH 3 conditions (achieved by the addition of H₃PO₄) and the
207 presence of tBuOH (10 mM) as •OH scavenger, in order to favour direct ozone reactions
208 and avoid the presence of this radical in the medium. The other round of experiments was
209 conducted at pH 10 (achieved by the addition of NaOH) to favour the formation of TPs
210 from hydroxyl radical oxidation. All solutions were prepared with Milli-Q water to avoid
211 interferences and facilitate the identification of TPs. Experiments were conducted by
212 adding different aliquots (1.5-5 mL) of the ozone stock solution to glass vials containing
213 the above-mentioned solutions spiked with CLO (40 µM). The total reaction volume was
214 25 mL. After complete consumption of ozone, samples were withdrawn and later used
215 for TPs determinations.

216

217 The identification of CLO transformation products was carried out by Liquid
218 Chromatography-Mass Spectrometry (LC-MS). For that purpose, an 1100 HPLC coupled
219 to a 6210 TOF mass spectrometer, all provided by Agilent, was employed. Both positive
220 and negative electrospray ionization was used to collect MS data in full scan mode (25-
221 1100 m/z). The separation of chemical species was achieved by similar conditions to
222 those employed in HPLC-DAD determinations.

223

224 A preliminary toxicity assessment was conducted to provide insights into the risks of
225 CLO and its transformation products. To that purpose, the Ecological Structure-Activity
226 Relationships (ECOSAR) model (U.S. EPA, 2022) was used for the ecotoxicological
227 screening of both, parent compound and TPs.

228

229 **3. Results and discussion**

230

231 *3.1. Kinetics of CLO abatement*

232

233 It is well-known that the ozone treatment to remove an aqueous compound can take place
234 simultaneously in two pathways: direct reaction by molecular ozone and indirect reaction
235 by hydroxyl radical (Hoigné & Bader, 1976). Therefore, since both O₃ and •OH can react
236 with the pesticide clothianidin, directly and indirectly, respectively, the ozonation
237 reaction kinetics can be expressed as a second-order (first-order concerning each reactant)
238 as follows:

239

$$-\frac{d[CLO]}{dt} = k_{CLO, O_3} \cdot [O_3][CLO] + k_{CLO, \bullet OH} \cdot [\bullet OH][CLO] \quad (1)$$

240

241

242 in which k_{CLO, O_3} and $k_{CLO, \bullet OH}$ are the second-order reaction rate constants of CLO with
243 ozone and $\bullet OH$ radical, respectively. In this regard, the first step in an ozonation kinetic
244 study is to separately determine the kinetics of each reaction outlined above (Beltrán et
245 al., 2000).

246

247 3.1.1. Rate constant for reaction with O_3

248

249 Several methods can be used for measuring the direct ozonation rate constant, including
250 pseudo-first-order reaction (either reactant or ozone is in excess) or competition kinetics
251 (Wen et al., 2011). At first, competition kinetics experiments were performed by using
252 sulfamethoxazole and metoprolol as reference compounds due to their high ($\sim 2.0 \cdot 10^6 \text{ M}^{-1}$
253 s^{-1}) and moderate ($\sim 2.0 \cdot 10^3 \text{ M}^{-1} \text{ s}^{-1}$) reactivity with ozone, respectively (Jin et al., 2012;
254 Zoumpouli et al., 2020). The outcomes suggested neither competitor was properly valid
255 since CLO appeared to react slowly with O_3 in both cases.

256

257 Since the reaction rate constant was suspected to be relatively low (i.e., $\leq 100 \text{ M}^{-1} \text{ s}^{-1}$),
258 CLO concentration was monitored directly over time in experiments with a significant
259 excess of ozone, thus guaranteeing a steady O_3 concentration throughout the experiment.
260 Besides, the presence of t-BuOH allowed minimising the $\bullet OH$ reaction. Under these
261 conditions, the reaction between O_3 and CLO was assumed to follow a pseudo-first-order
262 kinetic model. Hence, the pseudo-first-order rate constant (k'_{CLO, O_3}) could be determined
263 experimentally, and consequently, estimate the corresponding second-order rate constant.
264 In this sense, the rate of CLO degradation can be described as follows:

265

$$-\frac{d[CLO]}{dt} = k'_{CLO, O_3} \cdot [CLO] \quad (2)$$

$$k'_{CLO, O_3} = k_{CLO, O_3} \cdot [O_3] \quad (3)$$

266

267 Since O₃ concentration remains constant, rearranging Eq. (3) in Eq. (2), and integrating
268 the latter, the ensuing equation can result in a simplified form:

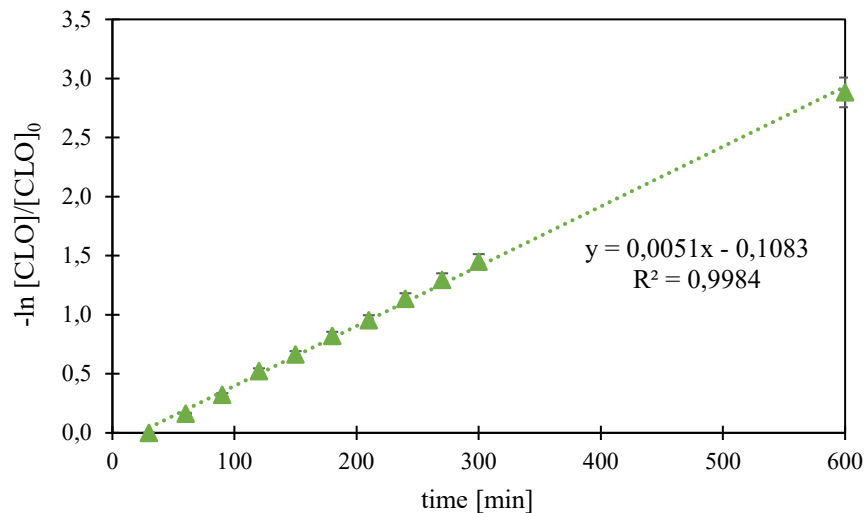
269

$$- \ln \frac{[CLO]_t}{[CLO]_0} = k'_{CLO, O_3} \cdot t \quad (4)$$

270

271 According to this expression, a linear dependence is achieved by plotting the natural
272 logarithm of the relative residual CLO concentration vs time. The slope of this function
273 would represent the pseudo-first-order rate constant for the reaction between O₃ and CLO.
274 Figure 1 shows the experimental results obtained, in which the linear correlation
275 coefficient ($R^2 > 0.99$) indicated that ozone oxidation for CLO degradation reaction fitted
276 the kinetic model reasonably well. Once the pseudo-first-order constant value was
277 established, corresponding with $5.1 \cdot 10^{-3} \text{ s}^{-1}$, the second-order rate constant for CLO
278 reacting with O₃ could be simply calculated. Under pH 7 conditions, and working with a
279 steady ozone concentration value of $4.9 \cdot 10^{-5} \text{ M}$ (average of initial and final values, with
280 a decrease of less than 3% during the experiment), the second-order rate constant
281 (k_{CLO, O_3}) for the reaction between CLO and O₃ was determined to be $102.64 \pm 4.36 \text{ M}^{-1}$
282 s^{-1} .

283



284

285 **Figure 1.** Determination of pseudo-first-order rate constant (k'_{CLO, O_3}) for the reaction between CLO and
286 molecular ozone. Conditions: $[CLO]_0 = 104 \mu\text{M}$, $[O_3]_0 = 49.3 \mu\text{M}$, $[t\text{-BuOH}]_0 = 10 \text{ mM}$, pH 7, temperature
287 $= 20.5 \pm 1 \text{ }^\circ\text{C}$.

288

289 Based on the abovementioned results and according to the categorization proposed by
 290 Lee and co-workers for the O₃ reactivity of micropollutants, the reaction rate between
 291 CLO and molecular ozone could be classified as moderate (Y. Lee et al., 2013).
 292 Furthermore, Table 1 compares different second-order rate constants values determined
 293 in previous studies for the reaction between other neonicotinoids and ozone. Considering
 294 the low kinetics between ozone and both pyridinic and chlorothiazole ring, observed
 295 differences in the second-order rate constants between CLO and the rest of neonicotinoids
 296 should be due to the different reactivity of amino groups. Thus, the two secondary amines
 297 in CLO would provide two possible reactive sites to O₃, whereas only one would be
 298 present in the case of acetamiprid. In addition, the cyano- group in that case may
 299 contribute to the observed suppression in ozone reactivity. Imidacloprid and
 300 thiamethoxam, on their part, have a secondary and a tertiary amine, on one hand, and two
 301 tertiary amines, on the other. However, even if the extra alkyl groups in tertiary amines
 302 contribute to increased electron density, potential steric effects which are comparatively
 303 reduced in the case of CLO may explain the drop in reactivity of both compounds (von
 304 Sonntag & von Gunten, 2012).

305
 306

Table 1. Second-order rate constants of different neonicotinoid pesticides with molecular ozone.

| Compound | k_{O3} [M⁻¹ s⁻¹] | k_{•OH} [M⁻¹ s⁻¹] | Reference |
|-----------------|---|--|---------------------------------|
| Clothianidin | 102.6 | 3.7·10 ⁹ | This work |
| Thiamethoxam | 15.4 | 3.9·10 ⁹ | (Wang et al., 2020) |
| Imidacloprid | 10.9 | 2.9·10 ⁹ | (Chen et al., 2019) |
| Acetamiprid | 0.25 | 2.1·10 ⁹ | (Cruz-Alcalde et al., 2017b) |

307

308 It is important to note that CLO presents a pK_a value of 11.09, so under the typical near-
 309 neutral working pH conditions (i.e., pH 6-8), molecules are still in their protonated form.
 310 In more alkaline conditions (especially when pH > pK_a), the amine group of CLO
 311 molecules would be in their non-protonated form, and this might favour the ozone
 312 oxidation mechanism (von Sonntag & von Gunten, 2012). In addition, the electron-
 313 withdrawing moieties of CLO, such as -Cl, -S- or -NO₂, would still have a negative impact
 314 on the electron density, thus leading to a considerable decrease in the rate constant (von
 315 Gunten, 2003). However, as mentioned these are not representative conditions of

316 wastewater ozonation. In any case, this is not the case for •OH radical since it is much
317 less selective and can attack a larger amount of CLO molecular sites.

318

319 3.1.2. Rate constant for reaction with •OH

320

321 For $k_{CLO,•OH}$ determination, since measuring •OH directly cannot be possible in ozonation
322 processes (Chelme-Ayala et al., 2011), competition kinetics were carried out in UV/H₂O₂
323 and UV systems by using *p*CBA as a competitor agent. This choice was due to its rapid
324 reaction with •OH radicals ($k_{pCBA,•OH} = 5.0 \cdot 10^9 \text{ M}^{-1} \text{ s}^{-1}$) but very slow with O₃ (k_{pCBA, O_3}
325 = $0.15 \text{ M}^{-1} \text{ s}^{-1}$) (von Sonntag & von Gunten, 2012). Apart from •OH radicals, the CLO
326 abatement through UV/H₂O₂ treatment also involves direct photolysis (Ikehata & El-Din,
327 2005). Therefore, its contribution must be previously determined and afterwards
328 subtracted from the observed pseudo-first-order rate constant in the UV/H₂O₂ process. In
329 this way, the second-order rate constant of CLO reacting with •OH could be properly
330 estimated as indicated in Eq. (5).

331

$$k_{CLO,•OH} = \frac{k_{CLO} - k_{CLO,P}}{k_{pCBA} - k_{pCBA,P}} \cdot k_{pCBA,•OH} \quad (5)$$

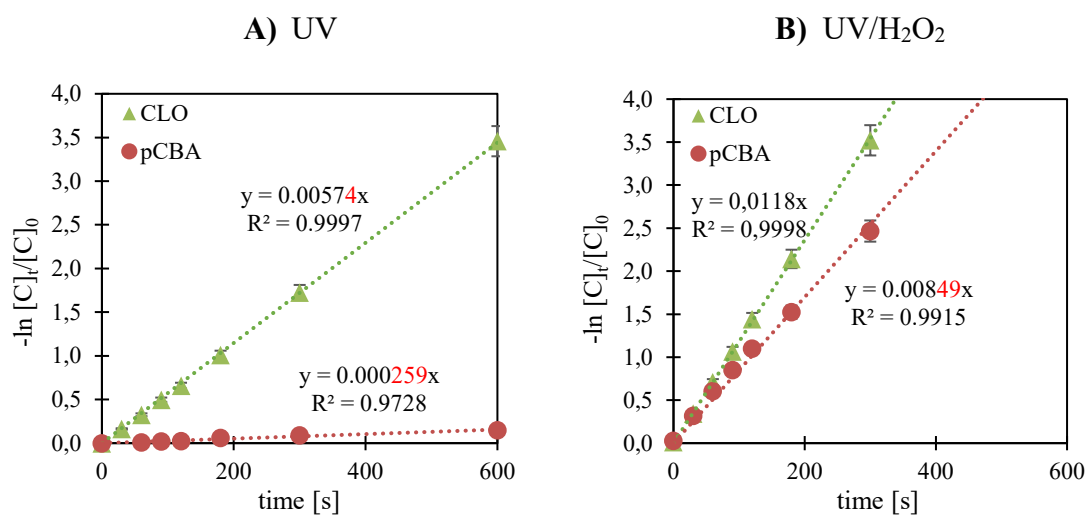
332

333 Where $k_{CLO,•OH}$ and $k_{pCBA,•OH}$ are the second-order rate constants for CLO and *p*CBA
334 with •OH, $k_{CLO,P}$ and $k_{pCBA,P}$ correspond to the first-order rate constants for direct
335 photolysis of CLO and *p*CBA, and lastly, k_{CLO} and k_{pCBA} represent the observed pseudo-
336 first-order rate constant of CLO and *p*CBA also in the UV/H₂O₂ treatment, respectively.
337 More details about the followed methodology can be found elsewhere (Porcar-Santos et
338 al., 2022).

339

340 Figure 2 shows the results of competition tests performed to determine the rate constant
341 for the reaction between CLO and •OH. Concerning only about UV system, as depicted
342 in Figure 2.A, the first-order rate constant attributed to direct photolysis of CLO
343 ($k_{CLO,P}$) was found to be $5.74 \cdot 10^{-3} \text{ s}^{-1}$, whereas for *p*CBA ($k_{pCBA,P}$) was $2.59 \cdot 10^{-4} \text{ s}^{-1}$.
344 These results indicated that the contribution of direct photolysis to the overall degradation
345 of CLO and *p*CBA by UV/H₂O₂ treatment was around 48% and 3%, respectively.

346



347 **Figure 2.** Linear dependence between the natural logarithm of relative concentration and reaction time of
 348 CLO with *p*CBA for both systems. A) CLO and *p*CBA degradation by UV. B) CLO and *p*CBA degradation
 349 by UV/H₂O₂. Conditions: [CLO]₀ = 1 μM, [*p*CBA]₀ = 1 μM, [H₂O₂]₀ = 0.28 mM, pH 7, temperature = 20
 350 ± 1 °C.

351

352 In contrast, as can be seen from the results in Figure 2.B, the pseudo-first-order rate
 353 constants for CLO and *p*CBA in the UV/H₂O₂ system were found to be an order of
 354 magnitude higher, corresponding in this case to average values of $1.18 \cdot 10^{-2} \text{ s}^{-1}$ and
 355 $8.49 \cdot 10^{-3} \text{ s}^{-1}$, respectively. Hence, by knowing the observed rate constants values and the
 356 value of $k_{pCBA, \bullet OH}$, the second-order rate constant for the reaction of CLO and $\bullet OH$
 357 ($k_{CLO, \bullet OH}$) was finally determined to be $(3.69 \pm 0.14) \cdot 10^9 \text{ M}^{-1} \text{ s}^{-1}$. Comparing it with rate
 358 constants published in literature, previous studies reported values within the same order
 359 of magnitude ($\sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$) for the reaction of $\bullet OH$ radicals and several pesticides,
 360 including neonicotinoids (see Table 1 and Chelme-Ayala et al., 2010, 2011; Cruz-Alcalde
 361 et al., 2017a). Finally, by analysing the resulting values of $k_{CLO, \bullet OH}$ and k_{CLO, O_3} , it could
 362 be inferred that CLO oxidation by $\bullet OH$ was about 7 orders of magnitude faster than that
 363 by O₃. This confirms the unselective character of the $\bullet OH$ -mediated oxidation of CLO
 364 compared to that by ozone, while provides some indications about the relative importance
 365 of each pathway in the degradation of the pesticide during ozonation processes. However,
 366 and apart from reaction kinetics, the overall effectivity of CLO ozonation will also
 367 strongly depend on the properties of the water matrix to be treated.

368

369 3.2. CLO degradation in different water matrices

370

371 Additional experiments using O₃ and O₃/H₂O₂ were performed in complex water matrices
 372 to test the process efficiency in more realistic scenarios. The original wastewater (W1)
 373 employed came from the effluent of a membrane bioreactor (MBR) in the Gavà-
 374 Viladecans WWTP (Barcelona, Spain). Two other water matrices were also employed to
 375 assess how organic matter and alkalinity, the main oxidant scavengers present in the
 376 solution, affected the process efficiency. W2 consisted of a 1:1 dilution of W1 with
 377 ultrapure water, while W3 was prepared by adding sodium bicarbonate to W2 until
 378 alkalinity was equal to that of W1. Table 2 lists the main physicochemical characteristics
 379 of all three waters, whereas Figure 3 depicts the CLO degradation achieved in each water
 380 and the dose of oxidant employed.

381

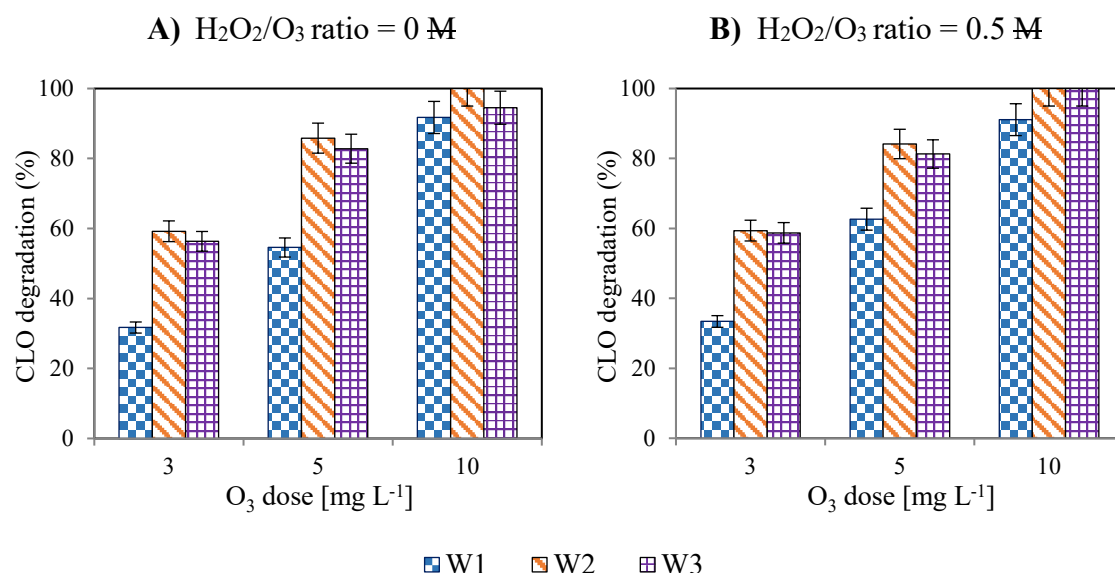
382 **Table 2.** Initial DOC, alkalinity parameter, and pH values for the three different water matrices employed.
 383 (W1: membrane bioreactor water, W2: W1 water – ultrapure water with a 1:1 dilution, W3: W2 with
 384 NaHCO₃ adjusted to the same initial level as W1.)

| Water matrices | Initial DOC [mg C L ⁻¹] | Initial alkalinity [mg CaCO ₃ L ⁻¹] | pH |
|----------------|--|---|-----|
| W1 | 6.7 | 233.2 | 7.8 |
| W2 | 3.4 | 116.6 | 7.5 |
| W3 | 3.4 | 233.2 | 7.6 |

385

386 From the outcomes shown in Figure 3.A, it can be observed that regardless of the O₃ dose
 387 applied, the lowest CLO degradation levels were obtained with W1. Compared to the
 388 other two water matrices, W1 presents the highest initial values for both DOC and
 389 alkalinity (see Table 2). Hence, together with the relatively low O₃ oxidation kinetics, this
 390 could be the main reason for the lower CLO degradation achieved (Chelme-Ayala et al.,
 391 2011). However, increasing the O₃ dose applied, from 3 mg L⁻¹ to 10 mg L⁻¹, led to a
 392 considerable upsurge in the CLO degradation with W1, specifically from 31.70% to
 393 91.73%. In contrast, with the other two water matrices, such changes were not as abrupt,
 394 although the same developing trend was also followed in both situations with increasing
 395 O₃ dosages. In this regard, the largest CLO degradations were obtained with W2, followed
 396 closely by W3. From Table 2, it can be observed that the principal difference between
 397 both matrices is the alkalinity content. Therefore, when comparing these results with
 398 those obtained with W1, it could be concluded that organic matter significantly exerted a
 399 higher effect on the scavenging of oxidants than carbonate alkalinity (Elovitz et al., 2000).

400 This is consistent with previous works since organic matter not only reacts with the
 401 unselective hydroxyl radical, but electron-rich fractions of DOC can also undergo
 402 oxidation by ozone (von Sonntag & von Gunten, 2012). By contrast, carbonate and
 403 bicarbonate anions can scavenge $\bullet\text{OH}$ but do not interact with O_3 . In fact, because of the
 404 $\bullet\text{OH}$ scavenging, these species indirectly reduce the rate of ozone decomposition.
 405



406
 407 **Figure 3.** CLO degradation in different water matrices with A) O_3 alone and B) molar $\text{H}_2\text{O}_2/\text{O}_3$ ratio of 0.5.
 408 (W1: membrane bioreactor water, W2: W1 water – deionized water with a 1:1 dilution, W3: W2 with
 409 NaHCO_3 adjusted to the same initial level as W1). Conditions: $[\text{CLO}]_0 = 100 \mu\text{g L}^{-1}$, pH 7, temperature =
 410 $20 \pm 1 \text{ }^\circ\text{C}$.

411
 412 Concerning experiments in the presence of hydrogen peroxide (molar $\text{H}_2\text{O}_2/\text{O}_3$ ratio of
 413 0.5), results in Figure 3.B indicated a similar pattern to those obtained in single ozonation
 414 tests. Only for W1, small variations of around 5% and 13% existed for dosages of 3 and
 415 5 mg L^{-1} , respectively. Furthermore, in W3 and 10 mg L^{-1} ozone, complete CLO removal
 416 was achieved (i.e., equivalent to an enhancement of around 5.5%). In any case, adding
 417 H_2O_2 did not lead to significant variations in the CLO degradation results, even though
 418 this reagent may theoretically increase the radical yield from ozone decomposition.
 419 However, in waters with relatively high DOC content (i.e., $> 1 \text{ mg DOC/L}$) the ozone
 420 decomposition and subsequent $\bullet\text{OH}$ formation is dominated by organic matter, which acts
 421 as both inhibitor and promotor of the process through faster reactions with this oxidant.
 422 The potential, extra formation of $\bullet\text{OH}$ due to H_2O_2 addition, involving significantly

423 slower reactions, is therefore masked by the predominant scavenging effect over hydroxyl
 424 radical exerted by organic matter (Acero & von Gunten, 2001).

425

426 According to the current knowledge of ozone oxidation, oxidant doses of up to 1 mg
 427 O₃/mg DOC are generally applied for micropollutants removal from wastewater (Y. Lee
 428 et al., 2013; Sgroi et al., 2014). Larger doses lead to increased costs and, in general,
 429 significant formation of oxidation byproducts (e.g., bromate). According to the results of
 430 this work, only abatements in the range of 50-80% would be attained by employing doses
 431 between 0.6 and 1 mg O₃/mg DOC, while significantly higher (and probably unpractical)
 432 dosages (i.e., up to 2 mg O₃/mg DOC) would be necessary to remove >80% of
 433 clothianidin in some of the tested waters. In such cases, therefore, additional treatments
 434 would be required for complete or near-complete removal of this pollutant.

435

436 3.3. Reaction intermediates and potential mechanisms

437

438 TPs generated during the ozonation of CLO solutions were investigated by HPLC-TOF-
 439 MS. Overall, five TPs were detected. Corresponding experimental and exact m/z in
 440 positive and negative ionization mode, suggested formulas and the experiments in which
 441 these species were detected (i.e., O₃-based oxidation and/or •OH-based oxidation) are
 442 shown in Table 3. For the assignment of molecular formulas, both the number of atoms
 443 and the mass error were considered in the final proposal. In all cases, only a candidate
 444 was left after discarding all suggestions in which the number of atoms was not reasonable,
 445 or the mass error was above 5 ppm (absolute value).

446

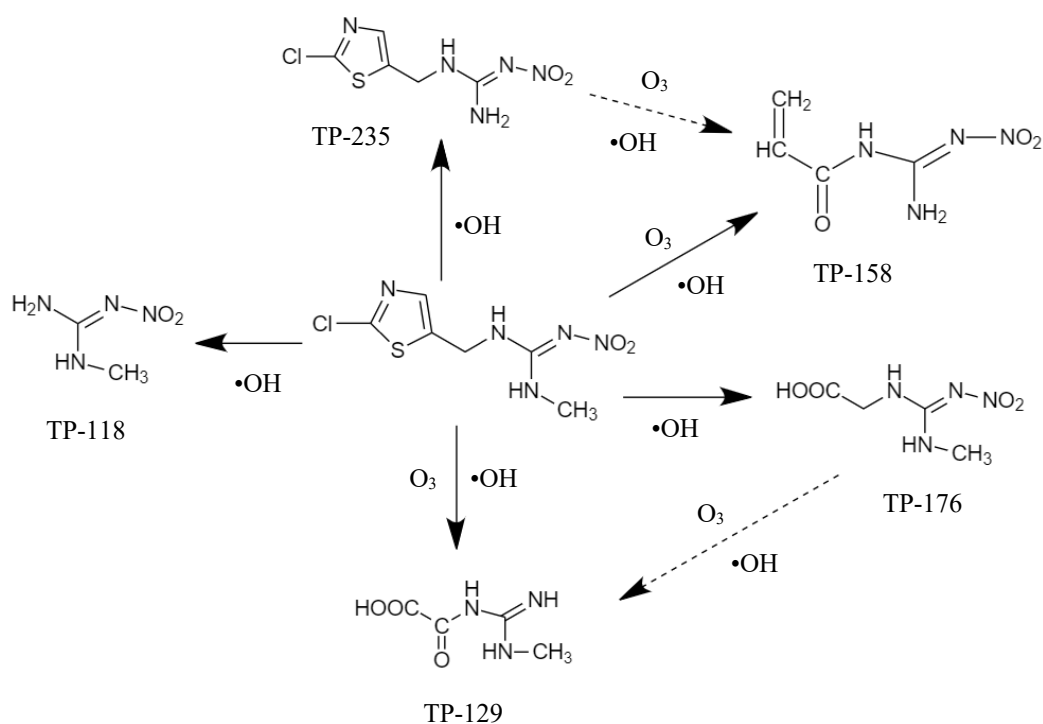
447 **Table 3.** Detected CLO-TPs, experimental and exact masses, suggested formulas, and species involved.

| Compound | Experimental mass | | Exact mass | | Formula [M] | O ₃ /•OH |
|----------|--------------------|--------------------|--------------------|--------------------|---|---------------------|
| | (m/z) | | (m/z) | | | |
| | [M-H] ⁻ | [M+H] ⁺ | [M-H] ⁻ | [M+H] ⁺ | | |
| CLO | 248.0019 | 250.0160 | 248.0014 | 250.0160 | C ₆ H ₈ ClN ₅ O ₂ S | O ₃ /•OH |
| TP-235 | 233.9856 | - | 233.9858 | - | C ₅ H ₆ ClN ₅ O ₂ S | •OH |
| TP-176 | 175.0471 | 177.0621 | 175.0473 | 177.0618 | C ₄ H ₈ N ₄ O ₄ | O ₃ /•OH |
| TP-158 | 157.0366 | - | 157.0367 | - | C ₄ H ₆ N ₄ O ₃ | O ₃ /•OH |
| TP-129 | - | 130.0614 | - | 130.0611 | C ₄ H ₇ N ₃ O ₂ | •OH |

448

449 According to the proposed molecular formulas and the chemistry of ozone and hydroxyl
450 radical oxidation reactions, the degradation pathways schematized in Fig. 4 are proposed
451 for CLO oxidation by ozone-based processes. TP-235 was suggested to be N-(2-
452 Chlorothiazol-5-ylmethyl)-N'-nitroguanidine, which would result from the loss of the
453 methyl group attached to the guanidine moiety of clothianidin. This degradation step
454 might presumably start by hydroxyl radical H-abstraction at the methyl group attached to
455 the secondary amine, followed by further oxidation by •OH. Previous works on the
456 oxidation of compounds presenting N-containing groups have shown how this pathway
457 is possible. For instance, Cruz-Alcalde and co-workers suggested a similar mechanism as
458 the explanation for observed tertiary amine demethylation in acetamiprid. Instead, and
459 related to this mechanism, if an initial attack by •OH took place at the adjacent methylene
460 group this would result in the breakage of the molecule at that point (Cruz-Alcalde et al.,
461 2017b). This is in fact what we suggest leading to N-methyl-N'-nitroguanidine (i.e., TP-
462 118) formation in the present work, which constitutes another independent (primary)
463 pathway for CLO degradation. Concerning this, Wang and co-workers suggested for the
464 breakage of the thiamethoxam to occur by initial H-abstraction in the methylene group
465 bridging the nitroguanidine and chlorothiazolyl moieties (Wang et al., 2020). In addition
466 to the previous pathways, clothianidin degradation might also happen via hydroxyl radical
467 addition to the C-C double bond at the chlorothiazole ring, resulting in the yield of TP-
468 176 as one of the products. The cleavage of the characteristic thiazole ring and subsequent
469 formation of carboxylated products, a process mediated by •OH radical addition, has been
470 previously reported as one of the main oxidation pathways of the insecticide
471 thiamethoxam during ozonation (Wang et al., 2020). Not surprisingly, in the present work
472 TP-176 was also found in experiments in which ozone was the main oxidant in the
473 medium. Addition of ozone to the C=C bond in the thiazole ring may result in site
474 hydroxylation, which upon further oxidation may yield a carboxylated TP. From the
475 resulting structure of TP-176, further oxidation via O₃ or •OH possibly led to the
476 formation of TP-129, a hypothesis which is compatible with the evolution of both TPs
477 with oxidant doses shown in Fig. 5, according to which TP-129 formation seems to be
478 steeper as the increase in TP-176 signal gradually drops. For this to occur, the formation
479 of a ketone and displacement of the nitro group is necessary. Regarding the ketonic
480 moiety, repeated hydroxylation of the methylene linking the carboxylic group with the

481 secondary amine might lead to the formation of a hydrated carbonyl (Cruz-Alcalde et al.,
 482 2017b). On the other hand, previous works on the oxidation of clothianidin and
 483 thiamethoxam by ozone-based processes and other systems involving hydroxyl radicals
 484 reported the formation of guanidine moieties from parent nitroguanidine (Meijide et al.,
 485 2016; Šojić et al., 2012). Finally, TP-158 is proposed to be the result of amine
 486 demethylation plus thiazole ring cleavage at S- and N- adjacent carbons and oxidation of
 487 the methylene group linking the thiazole ring with the secondary amine in the precursor
 488 molecule. Based on this, TP-158 could be a product of further oxidation of both, CLO,
 489 and TP-235. The latter pathway would be compatible with the observed evolution of TP-
 490 235 and TP-158 with the applied oxidant dose (see Fig. 5), in which a steeper growth is
 491 observed at the same time that TP-235 accumulation gradually stops to finally slightly
 492 decrease.
 493

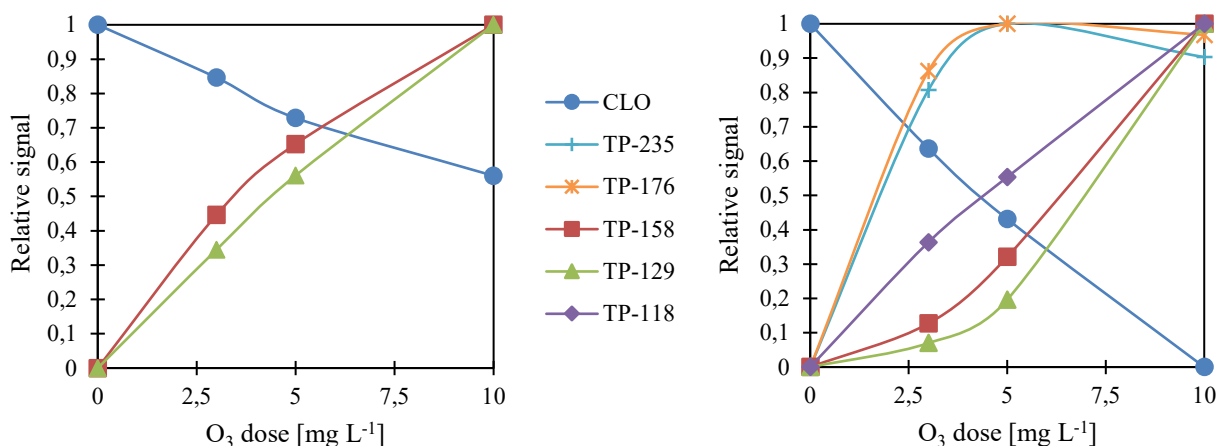


494
 495
 496

Figure 4. Proposed reaction pathways scheme for CLO degradation via O₃ and •OH during ozonation.

A) O₃ TPs

B) •OH TPs



497

498 **Figure 5.** Evolution of CLO and TPs as a function of the ozone dose. A) O₃ TPs experiment. Conditions:
 499 [CLO]₀ = 40 μM, [t-BuOH]₀ = 10 mM, pH 3, temperature = 20 ± 1 °C. B) •OH TPs experiment. Conditions:
 500 [CLO]₀ = 40 μM, pH 10, temperature = 20 ± 1 °C. C/C₀ relative signal is shown in the case of CLO, whereas
 501 A/A_{max} is plotted for TPs, where A is the area of the signal in the mass detector and A_{max} is the maximum
 502 value of this signal in each case.

503

504 In view of the proposed TPs, only one of the different formed CLO-TPs would lose their
 505 distinctive nitro-group from the guanidine moiety. This group is considered the main one
 506 responsible for making neonicotinoids more polar (and hence, more water-soluble) and
 507 active against target insects, as opposed to what happens with cyanoamidine-type
 508 neonicotinoids (Buszewski et al., 2019). Thus, the nitro metabolites are probably more
 509 toxic to non-target insects such as honeybees, in contrast to the desnitro metabolites (e.g.,
 510 TP-129) which present a greater selectivity and stronger affinity to bind to the mammalian
 511 nicotinic acetylcholine receptors (nAChRs) due to the positive charge distribution at their
 512 guanidine fraction (Thompson et al., 2020). However, not all these resultant compounds
 513 might have the same impact on different non-target organisms such as those found in
 514 water bodies, so ecotoxicity studies are necessary to elucidate the toxicity of TPs formed
 515 upon CLO oxidation by ozone and hydroxyl radical.

516

517 3.4. Ecotoxicity assessment

518

519 As demonstrated in the preceding section, the formation of different chemical species
 520 during the CLO ozonation process may lead to pose some toxicological threats to aquatic
 521 organisms. For that reason, the ecotoxicity of CLO and its corresponding ozonation TPs
 522 were estimated by employing the Ecological Structure Activity Relationships (ECOSAR)
 523 program (U.S. EPA, 2022). This software provides a prediction of aquatic toxicity of

524 chemicals at three different trophic levels, both acute (short-term) and chronic (long-term
 525 or delayed). Estimations are based on structure-toxicity correlations constructed from
 526 previous experimental data (Gao et al., 2014). The results of the predicted acute and
 527 chronic (ChV) toxicity for CLO and detected TPs, expressed as LC₅₀ for fish and aquatic
 528 invertebrates (Daphnid) and EC₅₀ for green algae, are listed in Table 4.

529
 530

Table 4. Ecotoxicity results for CLO and its TPs after ozonation using ECOSAR Program.

| Proposed compound | Acute toxicity [mg L ⁻¹] | | | Chronic toxicity (ChV) [mg L ⁻¹] | | |
|-------------------|--------------------------------------|-----------------------------|---------------------------|--|----------------------|----------------------|
| | Fish (LC ₅₀) | Daphnid (LC ₅₀) | Algae (EC ₅₀) | Fish | Daphnid | Algae |
| CLO | 373 | 37.9 | 42.8 | 35.1 | 2.67 | 12.7 |
| TP-235 | 712 | 68.6 | 86.1 | 79.0 | 4.59 | 24.5 |
| TP-176 | 1.34·10 ⁶ | 8.46·10 ⁴ | 2.46·10 ⁵ | 5.44·10 ⁵ | 3.74·10 ³ | 5.16·10 ⁴ |
| TP-158 | 4.77·10 ³ | 385 | 686 | 908 | 21.7 | 172 |
| TP-129 | 2.37·10 ⁴ | 1.68·10 ³ | 3.86·10 ³ | 6.69·10 ³ | 83.4 | 883 |
| TP-118 | 2.76·10 ³ | 227 | 389 | 494 | 13 | 99 |

531 * Predicted toxicity values classified according to the Globally Harmonized System of Classification and
 532 Labelling of Chemicals (GHS). Highlighted in white - not harmful (LC₅₀/EC₅₀/ChV > 100); highlighted in
 533 light blue - harmful (100 ≥ LC₅₀/EC₅₀/ChV > 10); highlighted in medium blue - toxic (10 ≥ LC₅₀/EC₅₀/ChV
 534 > 1); based on results, compounds considered as very toxic (LC₅₀/EC₅₀/ChV ≤ 1) were not present.

535

536 Based on the results listed above and according to the toxicity criteria set by the European
 537 Union in Directive 93/67/EEC (European Commission, 2003), the compounds TP-176,
 538 TP-158, TP-129 and TP-118 could be categorized as non-harmful in a short period of
 539 time (i.e., acute toxicity) for all three trophic levels. Contrarily, CLO and TP-235 would
 540 show a different trend to that previously reported, as they would remain harmless for fish,
 541 while increasing its toxicity and becoming harmful to daphnid and green algae.

542

543 On the other hand, by focusing on long-term toxicity, notable differences could be
 544 noticed. Both CLO and TP-235 remained at these harmful values but also included the
 545 trophic level of fish and increased for daphnid, for which they would be then considered
 546 toxic (1 < ChV ≤ 10). In addition, prolonged exposure to TP-158 and TP-129 would be

547 deemed only harmful to daphnids, whereas TP-118 would be also harmful to both
548 daphnids and green algae. The unique considerable exception was TP-176, which could
549 not be regarded as toxic or harmful, either in the short or long-term, for any considered
550 trophic level since their values were of a magnitude order within 10^3 - 10^6 mg L⁻¹. In
551 addition, it was also interesting to note that none of the resulting transformation products
552 (nor clothianidin) as such could be categorized as very toxic ($LC_{50}/EC_{50}/ChV \leq 1$).

553

554 From a structural point of view, the breakdown of the methyl radical from guanidine
555 moiety (see TP-235) did not significantly lower toxicity, so it could be speculated that
556 this was not the main responsible for the relatively high toxicity found for CLO.
557 Contrarily, when the chlorothiazole ring was removed from the parent molecule and the
558 rest of the structure remained unaltered as observed in TP-176, the acute toxicity
559 diminished substantially (see $LC_{50}/EC_{50}/ChV$ values in Table 4) to the point that this
560 compound would not be considered toxic for any of the species studied. Concerning the
561 rest of TPs detected, estimated toxicity was found between values calculated for CLO and
562 TP-176, probably due to the presence of larger molecular remainders of the thiazole
563 moiety or comparatively more toxic groups. As a consequence, all those TPs were
564 estimated to be harmful against Daphnids, although only regarding chronic effects. No
565 toxicity (either acute or chronic) was estimated for the rest of species. Overall, the
566 ecotoxicity results suggested that CLO TPs seem to be less damaging than their precursor
567 for different aquatic organisms. From the ecotoxicological point of view, therefore, this
568 would imply that in general ozonation and other ozone-based oxidation processes could
569 be a feasible treatment alternative for the removal of CLO.

570

571 **Conclusions**

572

573 The kinetic studies disclosed a relatively moderate reactivity of clothianidin, both with
574 ozone and hydroxyl radical, since the second-order rate constant values were determined
575 to be 103 ± 4 M⁻¹ s⁻¹ and $(3.69 \pm 0.14) \cdot 10^9$ M⁻¹ s⁻¹, respectively. According to these
576 results, it is suggested that, in general, CLO abatement is mainly driven by •OH oxidation
577 (indirect reaction) rather than through O₃ attack (direct reaction). Results suggested that
578 an additional •OH source (i.e., the H₂O₂ dosed in the O₃/H₂O₂ system) did not seem to
579 appreciably improve the overall CLO degradation efficiency in real wastewater treatment.
580 In addition, it was proved that the key parameter responsible for hampering higher CLO

581 abatements was the DOC content of the wastewater -rather than alkalinity-. However, in
582 both scenarios, typical O₃ dosages seem to be not sufficiently powerful to attain a
583 complete removal, achieving only around 50-80% CLO abatement. Overall, five major
584 CLO-TPs were identified. All these were generated through •OH-based oxidation,
585 whereas only two (i.e., TP-176 and TP-158) involved additional O₃-based oxidation. The
586 acute and chronic toxicities of CLO, as well as its formed TP, were evaluated at three
587 different trophic levels. Results highlight that no CLO by-products surpassed the parent
588 compound concerning toxicity, and once the chlorothiazole ring is removed, the toxicity
589 (both acute and chronic) decreases considerably for the different aquatic organisms. Thus,
590 in view of the obtained results, it can be inferred that the ozonation process can be
591 considered a suitable and feasible treatment alternative for CLO (and associated toxicity)
592 removal.

593

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595

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599

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