

1 **Priority pesticides abatement by advanced water technologies: the case of**
2 **acetamiprid removal by ozonation**

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4 A. Cruz-Alcalde*, C. Sans, S. Esplugas

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6 Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry,
7 Universitat de Barcelona, C/Martí i Franqués 1, 08028 Barcelona, Spain. Tel:
8 +34934029789; fax: +34934021291

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10 *Corresponding Author: alberto.cruz@ub.edu

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12 **ABSTRACT**

13
14 With the aim of exploring treatment alternatives for priority insecticide acetamiprid
15 (ACMP) abatement, the removal of this compound from water by ozonation was studied
16 for the first time, paying special attention to the kinetic, mechanistic and toxicological
17 aspects of the process. The second order rate constants of reactions between ACMP and
18 both molecular ozone (O_3) and hydroxyl radicals ($OH\cdot$) were determined to be $0.25\text{ M}^{-1}\text{s}^{-1}$
19 and $2.1\cdot 10^9\text{ M}^{-1}\text{s}^{-1}$, respectively. On the basis of kinetic results, the degradation of
20 ACMP during ozonation could be well-explained by the reactivity of this pesticide with
21 $OH\cdot$. HPLC/MS analysis of the ozonated ACMP showed ACMP-*N*-desmethyl, 6-
22 chloronicotinic acid, *N*'cyano-*N*-methyl acetamidine and *N*'cyano acetamidine as the
23 major transformation products (TPs), all of them formed through amine α carbon
24 oxidation in combination with hydrolysis. Microtox bioassays revealed an increase in
25 the toxicity of the medium during ACMP ozonation process, followed by a decrease to
26 relatively low values. These changes could be attributed to the synergistic effects
27 between TPs as well as to the presence of toxic intermediate aldehydes. Even though
28 adopting strategies to further promote ozone decomposition to hydroxyl radicals
29 appears to be essential, ozonation can be an effective treatment process for ACMP
30 removal and associated toxicity abatement.

31
32 **KEYWORDS**

33

34 Acetamiprid, priority pesticides, ozonation, hydroxyl radical oxidation, reaction
35 mechanisms, toxicity assessment

36

37 **1. Introduction**

38

39 Since 2013 some regulations regarding the identification, monitoring and control of
40 priority substances/groups of substances in aquatic compartments have been published
41 [1,2]. For example, the 1st watch list of substances for Union-wide monitoring, Decision
42 2015/495/EU [3], promotes the study of alternative water and wastewater treatment
43 options aimed to remove these substances from aqueous resources. Several pesticides
44 belonging to different families are included as priority pollutants. One of these groups,
45 neonicotinoids, are nowadays one of the most employed class of pesticides [4]. They
46 offer insect selectivity, excellent physicochemical properties, wide spectrum of efficacy
47 and a relative safe use in comparison with other pesticide classes like
48 organophosphorus, carbamates or pyrethroids [5,6]. The widespread use of these
49 chemicals have resulted in their occurrence in all environment compartments, including
50 water [5]. According to previous studies, the presence of neonicotinoids in nature could
51 be harmful to a broad range of invertebrate [7] and also vertebrate [8] non-target
52 organisms. Regarding the risks for human health, several recent studies have associated
53 the chronic exposure to neonicotinoids with certain types of developmental disorders
54 like congenital heart defects (CHD) [9], neural tube defects (NTD) [10] and autism
55 spectrum disorder (ASD) [11].

56

57 (*E*)-*N*-(6-chloro-3-pyridylmethyl)-*N'*-cyano-*N*-methyleacetamide, better known as
58 acetamiprid (ACMP) is a pesticide belonging to neonicotinoid insecticides class. It is
59 one of the most applied insecticides nowadays, being the fourth most employed
60 neonicotinoid in USA [4] and representing more than a 10% of the total sales of this
61 group of insecticides in the last years [12]. China, one of the largest ACMP producers,
62 had in 2013 a production of 8000 tons of this insecticide, 5000 of which were exported
63 [13]. Because of its extensive usage, this micropollutant has been detected in surface
64 (20-380 ng L⁻¹ [14]; 2.7-59.3 ng L⁻¹ [15]; 2-410 ng L⁻¹ [16]; up to 41 µg L⁻¹ [17]) and
65 also wastewater (50 ng L⁻¹ [18]) samples worldwide. The presence of ACMP in the
66 environment can pose risks to human health. Based on a previous work by Kimura-
67 Kuroda [19], the European Food Safety Authority (EFSA) recently delivered a

68 Scientific Opinion concluding that chronic exposure to ACMP could affect neural
69 development and function in humans [20]. A more recent study associated the chronic
70 exposure to this insecticide with some adverse effects on human health, including
71 memory loss and finger tremors [21]. Moreover, ACMP has been demonstrated to
72 negatively affect other species like aquatic [22] and soil [23] microorganisms, as well as
73 beneficial insects [24,25]. However, despite its presence in water compartments pose a
74 serious threat to environmental and human safety, scientific literature regarding the
75 removal of ACMP by means of non-conventional treatment technologies is still
76 incomplete [1]. Regarding the use of Advanced Oxidation Processes (AOPs) for this
77 purpose, some studies concerning the application of Fenton-based treatments [26,27],
78 heterogeneous photocatalysis [28,29] and other related technologies like the innovative
79 low temperature plasma [30] have been published in the last few years, all of them
80 demonstrating their potential for efficiently remove ACMP from different water
81 matrices. However, no reports concerning the employment of ozone for ACMP
82 abatement have been found.

83

84 Ozone-based processes have demonstrated to have great potential for micropollutants
85 removal from water [31–36]. This technology is based on the strong oxidizing capacity
86 of ozone (O_3), which also yields hydroxyl radicals ($OH\cdot$) during ozone decay [37].
87 Although ozone and hydroxyl radicals can be effective in removing pollutants,
88 transformation products (TPs) which may also be toxic can be formed during ozonation.
89 It is important, therefore, to possess full knowledge of this process by studying reaction
90 kinetics, transformation products, and residual toxicity of the treated water.

91

92 The present work aimed, for the first time, to go in-depth with the fundamentals (i.e.,
93 reaction kinetics, transformation products and associated toxicity evolution) of ACMP
94 ozonation process. The objective was to determine the reaction kinetics of this pesticide
95 when reacting with both, molecular ozone and formed hydroxyl radicals, as well as to
96 elucidate the possible reaction pathways and potential negative effects of the resulting
97 transformation products from ACMP degradation.

98

99 **2. Materials and methods**

100

101 2.1. *Chemicals and reagents*

102

103 Acetamiprid and *p*-chlorobenzoic acid analytical standards, as well as potassium
104 indigotrisulfonate, were acquired from Sigma-Aldrich (Germany). Na₂HPO₄, NaH₂PO₄,
105 H₃PO₄ and acetonitrile were purchased from Panreac (Spain), and were all analytical
106 grade. Milli-Q water was produced by a filtration system (Millipore, USA). Pure
107 oxygen ($\geq 99.999\%$) was supplied by Abelló Linde (Spain).

108

109 In order to control the effects of side mechanisms like hydrolysis, adsorption or UV-Vis
110 photolysis on ACMP disappearance during ozonation experiments, several control
111 assays were performed. All runs were carried out in 250 mL closed glass beakers, with
112 initial ACMP concentrations of 1 mg L⁻¹. For hydrolysis and adsorption experiments,
113 the beaker was covered with aluminum foil in order to avoid the possible influence of
114 ambient radiation. The pH in hydrolysis tests was adjusted to a value of 2 or 7 by
115 adding adequate quantities of H₃PO₄ and Na₂HPO₄. For adsorption experiments, several
116 plastic materials (different types of silicones, PVDF and PTFE) usually employed in
117 laboratory were put in contact with the pesticide solution. In all experiments, the
118 medium was under stirring conditions. Samples were withdrawn at 0, 1, 5 and 24 h, and
119 analyzed by HPLC-DAD. Results showed that ACMP remained stable prior to oxidant
120 addition.

121

122 2.2. *Ozonation experiments*

123

124 All experiments in this work were performed by mixing aqueous ozone stock solutions
125 with aqueous stock solutions of ACMP. Ozone stock solutions (10-12 mg L⁻¹) were
126 prepared in a 1000 mL jacketed reactor by continuously bubbling a gaseous
127 ozone/oxygen mixture (~ 48 mg L⁻¹) into Milli-Q water at a rate of 40 L/h, using a
128 301.19 Sander Labor Ozonator (Germany). The medium was maintained at a
129 temperature of 10 \pm 1 °C. The O₃ concentration in aqueous phase was continuously
130 monitored by means of a Q45H/64 ozone probe (Analytical technology, US) connected
131 to a liquid recirculation stream. All kinetic and degradation experiments were performed
132 in triplicate, at a controlled temperature of 20 \pm 2 °C.

133

134 The second-order rate constant for the reaction between ACMP and molecular ozone
135 was directly determined under pseudo-first order conditions [38], with a 50-fold molar
136 excess of ozone with respect to the target compound. In order to avoid the presence of
137 hydroxyl radicals in the system, the reaction medium was adjusted to pH 2 by adding
138 adequate quantities of H_3PO_4 and Na_2HPO_4 [39]. Experiments were performed in a
139 closed 250 mL bottle, in which the headspace was almost removed in order to avoid
140 aqueous ozone losses. ACMP and ozone stock solutions were mixed to reach initial
141 reactant concentrations of 4 and 200 μM , respectively, and the medium was stirred for
142 10 seconds to obtain homogeneous conditions. Aliquots of 0.5 mL were withdrawn at
143 prefixed reaction times, and immediately quenched with 2.5 mL of an indigo solution.
144 These samples were finally employed to determine the dissolved ozone concentration
145 [40], as well as to quantify the remaining concentration of ACMP by HPLC-DAD.

146

147 Due to the fast reaction rates expected for the reaction between ACMP and indirectly
148 formed hydroxyl radicals, competition kinetics method [31,35] must be employed in
149 order to determine the corresponding second-order rate constant. The selected reference
150 was *p*-chlorobenzoic acid (pCBA), since the reactivity of this chemical with molecular
151 ozone is very low ($\leq 0.15 \text{ M}^{-1}\text{s}^{-1}$, [38]), whereas its reaction with hydroxyl radicals is
152 fast ($5 \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$, [41]). In order to guarantee the proper generation of $\text{OH}\cdot$ while
153 maintaining a relative stability of aqueous ozone, experiments were performed at pH 7
154 by adding adequate quantities of a $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$ buffer (1 mM). Reactions were
155 conducted employing a multi-reactor system, successfully used in several previous
156 works [32,42]: in a series of 25 mL vials containing 4 μM of ACMP and 4 μM of
157 pCBA, different doses (from 5 to 35 μM) of the ozone stock solution were injected and
158 mixed. Samples were taken when the total consumption of ozone was achieved (all
159 within 2 h). The residual concentrations of ACMP and pCBA were determined by
160 HPLC-DAD.

161

162 With the aim of demonstrating the relative contribution of each oxidant involved in
163 ozonation (i.e., molecular ozone and hydroxyl radicals) to ACMP degradation, two
164 additional sets of experiments were performed. Again, the multi-reactor methodology
165 was used. For direct reaction with ozone, each reaction vial (total volume of 25 mL)
166 contained 10 μM of ACMP and 25 mM of *tert*-butanol as radical scavenger. The pH of
167 the solution was adjusted to 7 by adding adequate quantities of a phosphate buffer (1

168 mM). For reaction involving both, the attack of ozone and hydroxyl radicals, a similar
169 procedure was followed but no scavenger was employed. In all experiments, ozone
170 doses between 5 and 175 μM were injected to each vial. Samples were withdrawn when
171 the total consumption of ozone was achieved (all within 2 h). Once the residual
172 concentration of ACMP was chromatographically determined, the corresponding
173 samples were frozen and later employed for TPs and toxicity determinations.

174

175 2.3. Analytical procedures

176

177 The concentrations of ACMP and pCBA were quantified by means of a high
178 performance liquid chromatograph (HPLC) equipped with a diode array detector
179 (DAD), all supplied by Agilent (1260 Infinity). The column employed was a
180 Teknokroma Mediterranea Sea18 (250 mm x 4.6 mm and 5 μm size packing). For
181 ACMP analysis, the mobile phase consisted on a 30:70 volumetric mixture of
182 acetonitrile and Milli-Q water acidified at pH 3 by the addition of H_3PO_4 . The flow rate
183 was maintained at 1.4 mL min^{-1} , and the detection wavelength was set to 250 nm. For
184 pCBA quantification, the mobile phase consisted on a 50:50 volumetric mixture of
185 acetonitrile and pH 3 Milli-Q water. The flow rate was set to 1 mL min^{-1} and the UV
186 detection was performed at 236 nm. The limits of detection (LODs) for ACMP and
187 pCBA were 0.018 and 0.029 μM , respectively.

188

189 With the aim of elucidating the ACMP degradation pathways given in ozonation
190 process, samples in which different ozone doses were applied were analyzed by Liquid
191 Chromatography-Mass Spectrometry (LC-MS). An Agilent 1100 HPLC coupled with a
192 G1969A LC/MSD-TOF mass spectrometer was employed. MS data were collected in
193 full scan mode (25-1100 m/z), employing positive electrospray ionization. The
194 separation of chemical species was achieved by operating with the following elution
195 program: a 5:95 volumetric mixture of ACN and Milli-Q (pH 3) was maintained as
196 initial mobile phase for 5 min; then, a linear gradient changed the eluent composition
197 from 5:95 to 30:70 in 5 min; the 30:70 mixture was maintained during the next 10 min
198 and, finally, a linear gradient returned back the eluent initial composition (5:95) in 5
199 min.

200 In order to assess the toxicity changes along the ACMP ozonation process, Microtox[®]
201 bioassays were performed. This method measures the inhibition of light emission of

202 bioluminescent bacteria *Vibrio fischeri* caused by the presence, in aqueous media, of
203 toxic compounds. The results of this assay are usually expressed as $EC_{50,15min}$, which
204 represents the percentage of sample dilution (% v/v) that causes a 50% reduction in
205 bacteria luminescence after 15 min of exposure. All the tests were carried out in
206 duplicate in a Microtox[®] M500 (Modern Water, UK) toxicity analyzer.

207

208 **3. Results and discussion**

209

210 *3.1. Kinetics of ACMP reactions with ozone and hydroxyl radicals*

211

212 Under the experimental conditions employed in these assays (i.e. pH 2), the half-life of
213 molecular ozone in pure aqueous solutions was observed to be more than 6 h, thus
214 evidencing that no radical chain reaction occurred. It was assumed, therefore, that
215 molecular ozone was the only oxidant in the reaction medium. Thus, the second-order
216 rate constant for the reaction between ACMP and O₃ could be calculated from Eq. 1,
217 being obtained by integrating the corresponding kinetic equation.

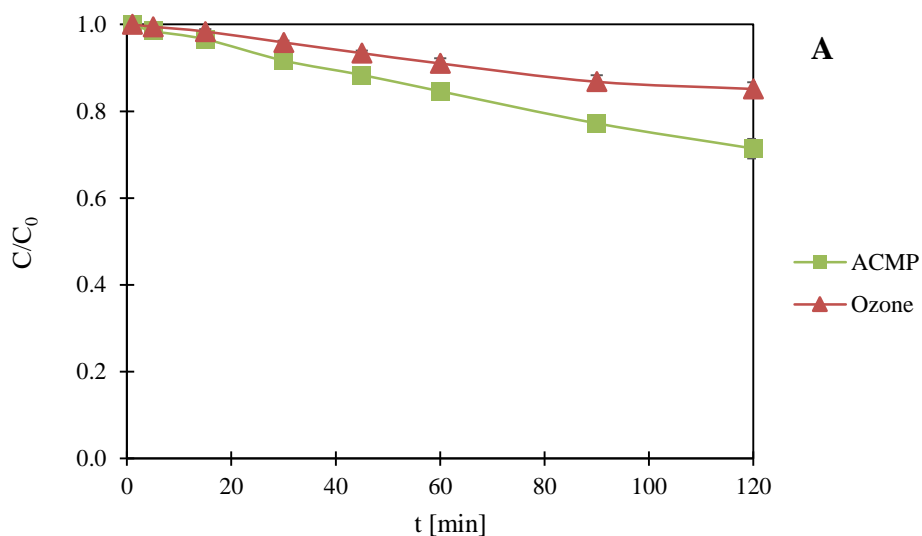
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$$-ln\left(\frac{[ACMP]}{[ACMP]_0}\right) = k_{MC,O_3} \int_0^t [O_3]dt \quad (1)$$

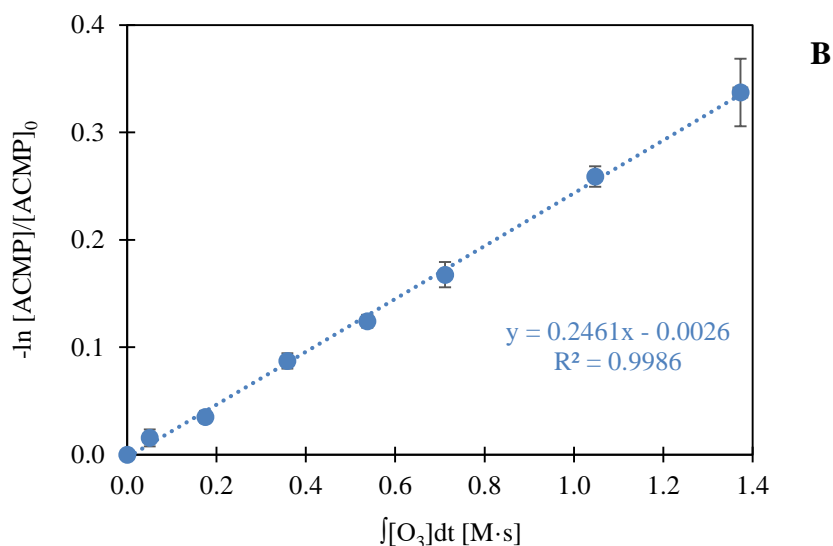
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220 By plotting the natural logarithm of the relative residual concentration of ACMP against
221 the ozone exposure, $\int [O_3]dt$, a linear relation was obtained. The slope of the function
222 corresponds to the second-order kinetic constant for the reaction between ACMP and
223 molecular ozone. Figure 1 shows the relative concentration of ACMP and ozone during
224 the experiment (A), as well as the logarithmic relative concentration of ACMP as a
225 function of $\int [O_3]dt$ (B). The ozone exposure was determined by calculating the area
226 under the ozone decay curve, employing the trapezoidal method of numerical
227 integration. k_{ACMP-O_3} was determined to be $0.25 \pm 0.02 \text{ M}^{-1}\text{s}^{-1}$.

228



229



230

231 Figure 1. Determination of second-order rate constant (k_{ACMP-O_3}) for the reaction of ACMP and ozone. A)
 232 Relative concentration of ACMP and ozone vs reaction time. B) Natural logarithm of the relative
 233 concentration of ACMP vs ozone exposure. Conditions: $[\text{ACMP}]_0 = 4 \mu\text{M}$, $[\text{O}_3]_0 = 200 \mu\text{M}$, pH 2,
 234 temperature = $20 \pm 2 \text{ }^\circ\text{C}$.

235

236 In the view of the above results it is clear that reactivity of ACMP towards direct ozone
 237 attack is very low, as expected from preliminary experiments. It is important to note that
 238 the determined value corresponds to ACMP deprotonated form, since the pKa value of
 239 this pesticide is 0.7 [43]. Therefore, the rate constant value should remain unaltered for
 240 higher pH values, including the ones exhibited by most water and wastewater real
 241 matrices.

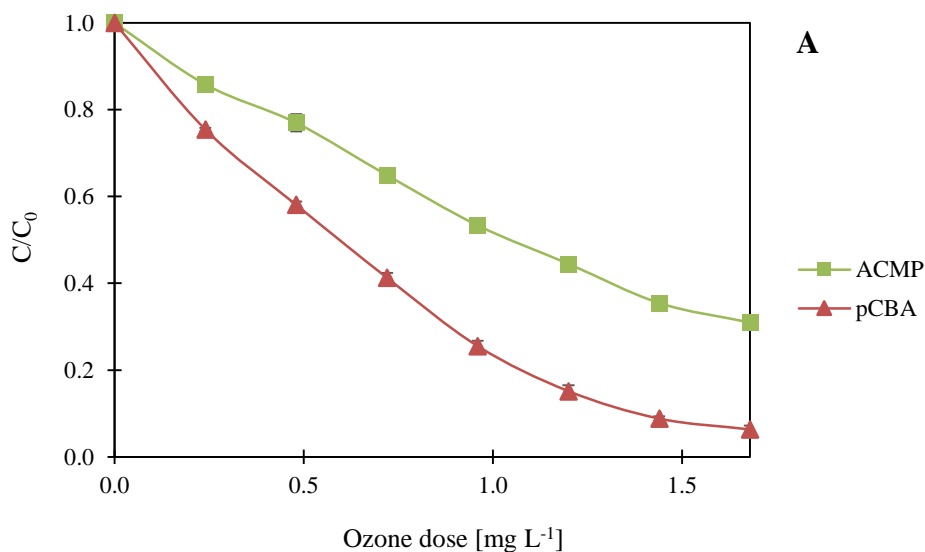
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243 Since the reactivity of ACMP and pCBA with molecular ozone is very low, and
 244 reactions between these chemicals and OH· were expected to be fast, it was assumed
 245 that depletion of both compounds under the employed experimental conditions (pH=7)
 246 was only due to OH· attack. Therefore, the second-order rate constant for the reaction
 247 between ACMP and OH· could be calculated from Eq. 2, being obtained by dividing the
 248 kinetic equations corresponding to reactions between OH· and both ACMP and pCBA.
 249

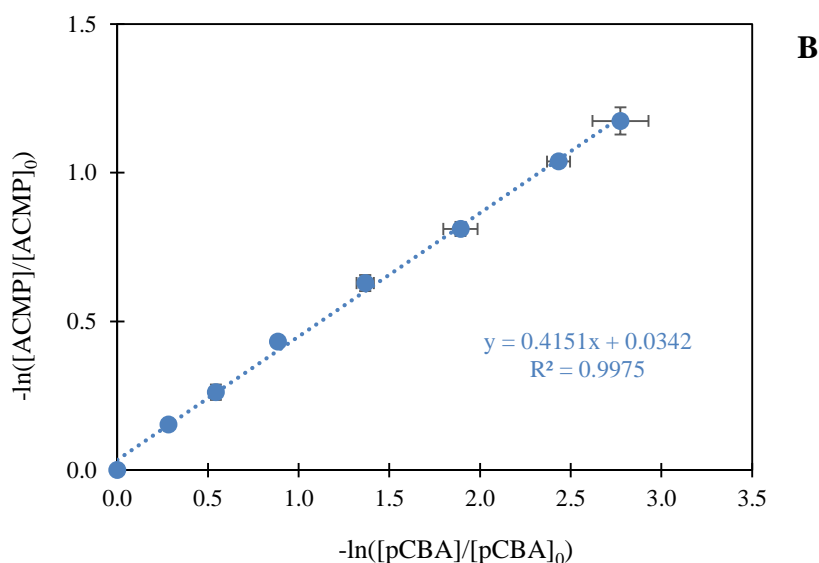
$$-\ln\left(\frac{[ACMP]}{[ACMP]_0}\right) = \frac{k_{ACMP,OH\cdot}}{k_{pCBA,OH\cdot}} \left(-\ln\left(\frac{[pCBA]}{[pCBA]_0}\right)\right) \quad (2)$$

250
 251 According to the above expression, a linear dependence between the natural logarithm
 252 of the relative ACMP concentration and the natural logarithm of the relative pCBA
 253 concentration was expected. The slope of this relationship represents the ratio between
 254 the second-order rate constants of OH· with target (ACMP) and reference (pCBA)
 255 compound, respectively. Figure 2 shows the relative concentration of both ACMP and
 256 pCBA as a function of the ozone dose (A), as well as the natural logarithm of ACMP
 257 relative concentration as a function of the natural logarithm of the relative pCBA
 258 concentration (B). A value of $(2.1 \pm 0.1) \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$ was finally determined for $k_{ACMP,OH\cdot}$.
 259 The high reactivity of ACMP with hydroxyl radicals is explained by the non-
 260 selective character of the oxidant [37] which readily undergo reactions with different
 261 points of organic molecules.

262



263



264

265 Figure 2. Determination of second-order rate constant ($k_{ACMP,OH\cdot}$) for the reaction of ACMP and $OH\cdot$ by
 266 competition kinetics. A) Relative concentrations of ACMP and pCBA as a function of the ozone dose. B)
 267 Natural logarithm of ACMP relative concentration vs natural logarithm of pCBA relative concentration.
 268 Conditions: $[ACMP]_0 = [pCBA]_0 = 4 \mu M$, pH 7, temperature = $20 \pm 2 \text{ }^\circ C$.

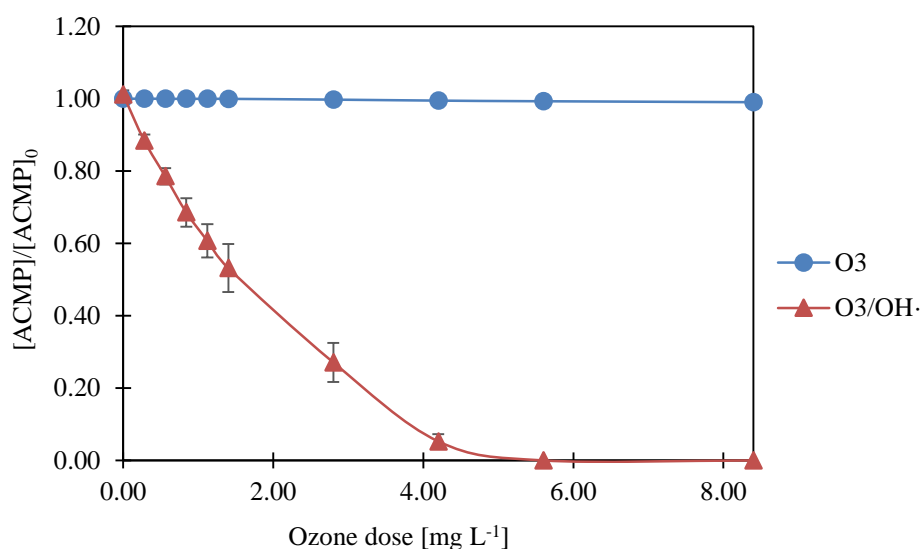
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270 3.2. ACMP degradation by ozone and hydroxyl radicals

271

272 It is well known that during ozonation, a compound can directly react with molecular
 273 ozone but also with hydroxyl radicals formed through O_3 decomposition [37]. With the
 274 aim of observing and comparing the removal of ACMP by both possible transformation
 275 routes, degradation experiments were conducted at pH 7 with and without the presence
 276 of a radical scavenger. Results are shown in Figure 3. Degradation by means of the
 277 direct reaction barely occurred, which was not surprising considering the extremely low
 278 rates exhibited by the reaction between ACMP and O_3 during the preceding kinetic
 279 runs. Besides, under the employed neutral pH conditions, ozone self-decomposition
 280 becomes relevant and therefore the stability of this oxidant in the medium is reduced
 281 with respect to more acidic conditions. For its part, indirect transformation through
 282 hydroxyl radicals demonstrated the effectiveness usually exhibited by this transient
 283 species in organics oxidation, and showed to be in total agreement with the findings of
 284 the previously mentioned studies dealing with the removal of ACMP by means of other
 285 AOPs [26,28]. With an ozone dosage of approximately 5.50 mg L^{-1} , the complete
 286 removal of ACMP was achieved.

287



288

289 Figure 3. Profile of ACMP degradation as a function of the ozone dose, for experiments with (O₃) and
 290 without (O₃/OH·) the presence of *tert*-butanol (25 mM). Conditions: [ACMP]₀ = 10 μM, pH 7,
 291 temperature = 20 ± 2 °C.

292

293 Considering the initial concentration of ACMP in degradation experiments, a
 294 normalized ozone dose of approximately 2.50 mg O₃/mg DOC (Dissolved Organic
 295 Carbon) was required to 100% eliminate ACMP under the studied conditions. This
 296 oxidant dosage, according to literature [45,46], would probably be considered expensive
 297 since doses up to 1 mg O₃/mg DOC are usually enough for disinfection and trace
 298 pollutant removal in drinking and wastewater treatment plants [45,46]. Because of that
 299 reason, and considering that ozone decomposition to hydroxyl radicals is the key of
 300 ACMP removal by ozonation, strategies aimed to further promote this indirect route
 301 should be pursued to make the process a competitive treatment option for waters
 302 contaminated by this compound. It is important to note, however, that since the process
 303 performance would always depend on water characteristics, like pH or inorganic and
 304 organic matter type and concentrations, the application in real matrices should be
 305 properly evaluated in future studies. With that purpose, experiments with real water
 306 matrices and pesticide concentrations should be performed. Another good option would
 307 be the employment of kinetic models based on the use of water specific information and
 308 the rate constants determined in this study [45,46].

309

310 3.3. Reaction intermediates and possible mechanisms

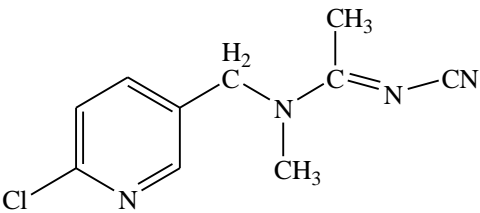
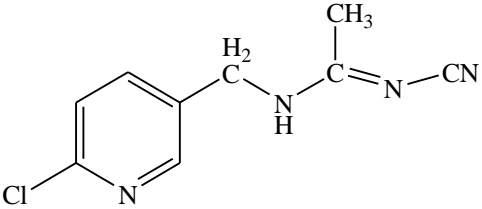
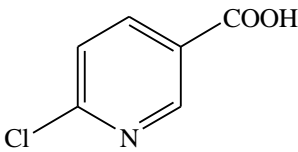
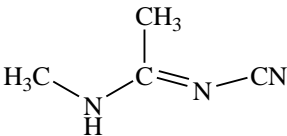
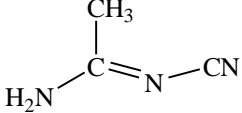
311

312 The identification of major TPs generated during ACMP ozonation was performed by
 313 means of LC-MS, being the corresponding chemical structures proposed on the basis of
 314 the detected masses. Since ozone was shown to be ineffective degrading ACMP
 315 molecules, it could be stated that all the detected species were reaction intermediates
 316 corresponding to products of OH· reactions, that is, the indirect reaction pathway. The
 317 molecular structures of the TPs that were identified are shown in Table 1.

318

319

Table 1. ACMP, detected TPs and corresponding molecular structures.

m/z	Name	Proposed structure
223 (m+1)	Acetamidrid (ACMP)	
209 (m+1)	ACMP-209 Acetamidrid- <i>N</i> - desmethyl	
158 (m+1)	ACMP-158 6-Chloronicotinic acid	
98 (m+1)	ACMP-98 <i>N</i> '-cyano- <i>N</i> - methyl acetamidine	
84 (m+1)	ACMP-84 <i>N</i> '-cyano acetamidine	

320

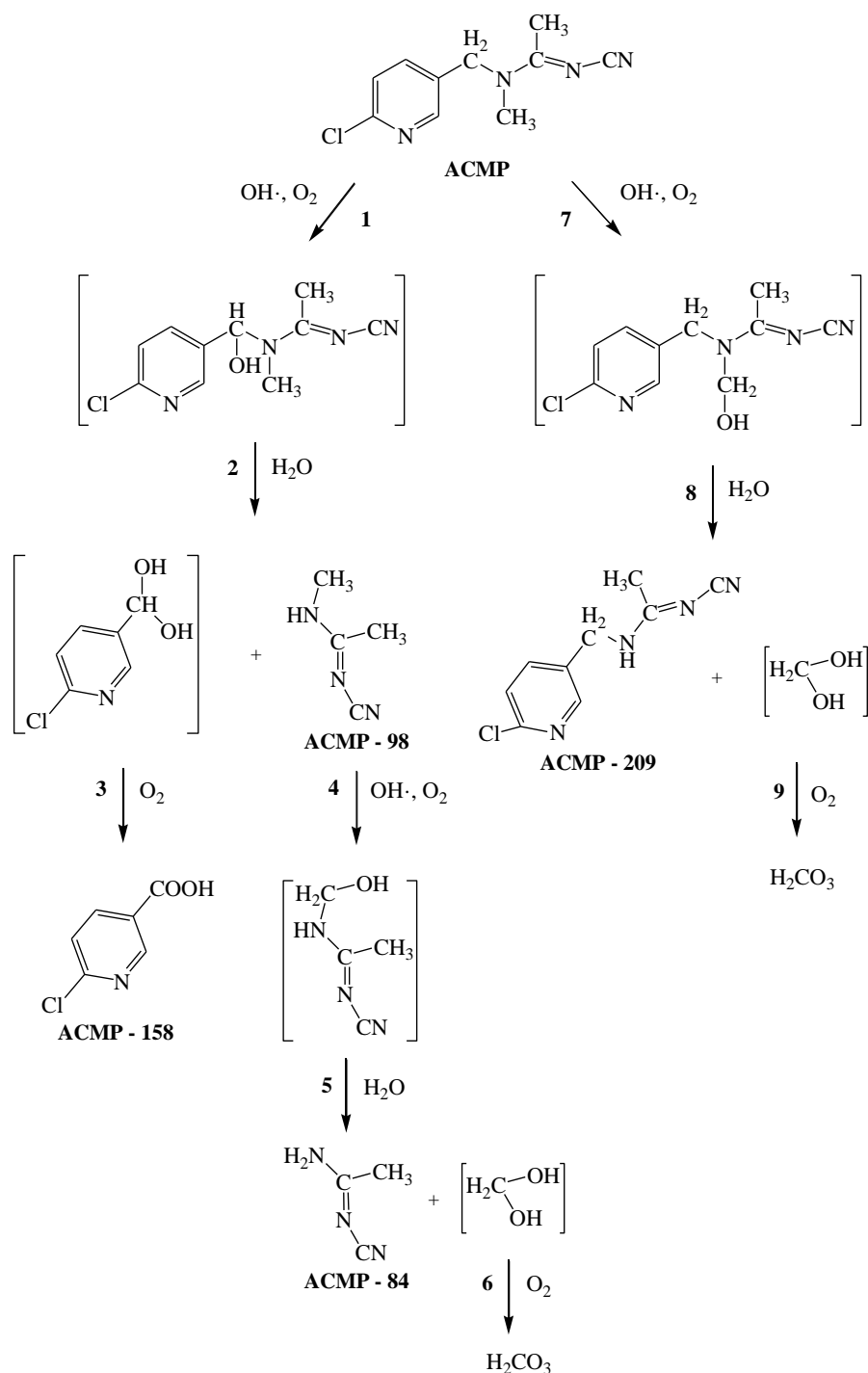
321 According to the detected structures and the experimental conditions employed in the
 322 study, the first stages of ACMP degradation during ozonation process could consist on a
 323 combination of OH· oxidation and fast hydrolysis of the metabolites generated during

324 the first step, as shown in Figure 4. The brackets in some of the proposed intermediates
325 indicate that these species could not be detected during the analysis, probably due to
326 their low concentration or fast tendency to undergo hydrolysis or become oxidized.

327

328 The identification of ACMP-158, ACMP-98 and ACMP-84 suggests that the initial
329 attack by hydroxyl radicals takes place at the methylene group (C α) of the ACMP
330 amine. After the fast H-abstraction carried out by OH \cdot , further oxidation of the α -
331 aminoalkyl radical by molecular oxygen in the presence of OH $^-$ yields the corresponding
332 α -hydroxymethylamine (reaction **1**). This mechanism is similar to the one reported by
333 Das et al. for trimethylamine OH \cdot -induced oxidation [47]. The hydrolysis of the
334 hydroxymethylamine (reaction **2**) would lead to the generation of *N*'-cyano-*N*-methyl
335 acetamide (ACMP-98) and 6-chloronicotinoid acid (ACMP-158). The latter, however,
336 would require a previous step which should involve the generation of the corresponding
337 aldehyde hydrate and its subsequent transformation to a carboxylic acid (reaction **3**),
338 being the latter step caused by the oxidizing conditions of the medium [48]. For its part,
339 further attack to ACMP-98 by hydroxyl radicals in the presence of O $_2$ (reaction **4**)
340 would result on the generation of its demethylated form, or *N*'-cyano acetamide
341 (ACMP-84), after the hydrolysis of the corresponding, previously formed
342 hydroxymethylamine (reaction **5**). Instead of at the methylene group, the initial H-
343 abstraction from ACMP structure can also take place at the methyl group of its amine
344 moiety (also an alpha C) (reaction **7**). Hydrolysis of the resulting hydroxymethylamine
345 (reaction **8**) would finally give ACMP-*N*-desmethyl (ACMP-209). It is interesting to
346 mention that the hydrated form of formaldehyde would be yielded as a side product of
347 ACMP-97 and ACMP-208 hydrolysis. Under the oxidizing conditions of the medium,
348 this compound could eventually be transformed and yield carbonic acid as final product
349 (reactions **6** and **9**).

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Figure 4. Proposed reaction pathways for ACMP degradation by OH· during ozonation process.

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The pesticide properties of ACMP are based on its nicotinoid structure, which mimics the vital neurotransmitter acetylcholine (ACh) by binding to the corresponding nicotinic acetylcholine receptor (*nAChR*) [6]. Due to the fact that this specific neural pathway is more abundant in insects than in warm-blooded animals, ACMP and those of its family (i.e. neonicotinoids) are more toxic to insects than to mammals [49]. Since the presence of the nicotinoid structure appears to be fundamental to maintain this selectivity against

360 pests, TPs ACMP-98 and ACMP-84 could have lost its ability to bind the insect
361 *n*AChRs and thus their insecticide features, which did not necessarily mean that these
362 side products were non-toxic. By the same argument, the intermediates ACMP-209 and
363 ACMP-158 (i.e. acetamiprid-*N*-desmethyl and 6-chloronicotinic acid) could still
364 maintain certain specificity in their pesticide action. However, in order to ensure a
365 proper interaction with the *n*AChRs and therefore a high selective action against insects,
366 it is also important for neonicotinoid species to possess an electronegative moiety on
367 their molecule to bind to the unique, positive charged amino acid residue present in the
368 nicotinic cholinergic receptor [49,50]. In relation with that, it has been found that nitro
369 or cyano substituents could be the most adequate electron-withdrawing moieties to
370 enhance the affinity between the pesticide and the receptor subsite [49,50]. Therefore, in
371 the present case it is expected for 6-chloronicotinic acid to present less affinity with
372 *n*AChRs and thus, an also less selective pesticide action than the exhibited by
373 acetamiprid-*N*-desmethyl, which would still keep the original cyano group of ACMP.

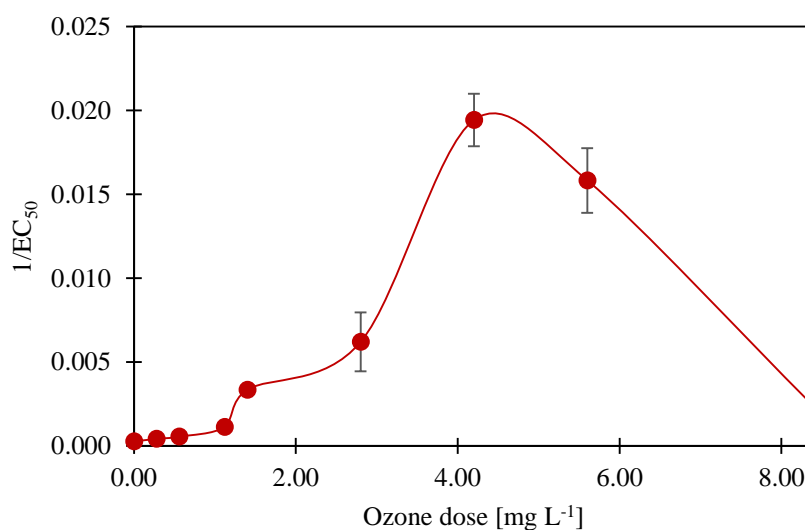
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375 3.4. Toxicity evolution during ACMP ozonation process

376

377 Due to the changes on the reaction medium composition, caused by the generation of
378 new products and the degradation of parent compound, ACMP ozonation process also
379 involved changes in the solution toxicity. The evolution of this property is represented
380 in Figure 6 by the $1/EC_{50}$ value for *Vibrio fischeri* assays, as a function of the ozone
381 dosage. It is important to remember that higher $1/EC_{50}$ values mean higher toxicities,
382 and vice versa. Initial EC_{50} value, that is, the effective concentration that inhibits a 50%
383 of the bacteria light emission, was determined to be 86 mg L⁻¹ (about 40 times the initial
384 concentration, in terms of sample dilution), which clearly represents a low toxicity to
385 non-target species. This value is lower than the one reported by Dell’Arciprete et al.
386 (129 mg L⁻¹), which was obtained by exposing bioluminescent bacteria to solutions with
387 different concentrations of ACMP. For the rest of samples, only EC_{50} in terms of
388 percentage of dilution (% v/v) could be determined since their compositions were
389 unknown. The toxicity of the medium notably increased for ozone doses above 3 mg L⁻¹,
390 reaching a maximum (EC_{50} 51.6%) for an O₃ dosage of approximately 4.50 mg L⁻¹.
391 The application of larger ozone doses resulted on significant toxicity abatement, as
392 observed in the $1/EC_{50}$ profile. A similar value than the starting one was practically
393 achieved for an ozone dose about 8.5 mg L⁻¹.

394 In previous works regarding ACMP degradation by oxidation processes in which
395 hydroxyl radicals and other ROS (Reactive Oxygen Species) were involved, an increase
396 in the medium toxicity was observed after the treatment with respect to the untreated
397 solution [48,51]. The same happened in a previous research regarding the photocatalytic
398 degradation of 6-chloronicotinic acid, one of the ACMP TPs detected in this work
399 (ACMP-158) [52]. Although the results in this study agree with the previous related
400 literature, a larger extent of the oxidation reaction in the present case with respect to the
401 preceding researches also led to the degradation of the intermediate species that caused
402 the observed increase in the medium toxicity.
403



404
405 Figure 5. Acute toxicity of the reaction medium during ACMP ozonation, as a function of the ozone dose.
406

407 The changes in toxicity observed during ACMP ozonation can be attributed, as in many
408 other works [33,34,52] to the generation of TPs more toxic than the parent compound,
409 as well as to the possible synergistic effects between initial and newly formed species
410 present in the reaction medium. Because of the relative loss in their pesticide selectivity,
411 it would be reasonable to consider ACMP-158, ACMP-98 or ACMP-84 as the most
412 suitable candidates leading to the bacteria bioluminescence inhibition, as *Vibrio fischeri*
413 could be considered as a non-target species. ACMP-209, for its part, should still
414 maintain a similar activity than the parent compound, and therefore a relative high
415 specificity against insects. However, ACMP-158 (6-chloronicotinic acid) has been
416 reported to be less toxic to *Vibrio fischeri* than ACMP itself [48], and no information
417 regarding the response of this bioluminescent bacteria under ACMP-98 and ACMP-84

418 exposure has been found. Given the scarcity of data on that topic, it was not possible to
419 attribute the increase in toxicity to the single presence of one of the detected TPs. In
420 addition to the possible synergistic effects between all the present species, as earlier
421 mentioned, it is possible that the observed changes were related to the presence of other
422 toxic intermediates that could not be identified during the LC-MS analyses. In the
423 preceding section it has been stated that aldehyde hydrate compounds (formaldehyde
424 and 6-chloronicotinaldehyde hydrates) could be involved in the ACMP degradation
425 pathway. Since these compounds are typically in equilibrium with their parent
426 aldehydes, and the latter have already proven to be highly toxic to Microtox[®] bacteria
427 (EC_{50} of 1.35 mg L⁻¹ for formaldehyde versus 89 mg L⁻¹ for ACMP determined in this
428 study) [36,53], that could constitute an alternative good explanation to the observed
429 increase in toxicity.

430

431 Considering the Microtox results, it is clear that ozonation applied to waters
432 contaminated by ACMP could cause an increase in the toxicity of the medium, at least
433 within a certain range of ozone doses. Since the employment of this treatment will
434 always pursue the complete depletion of the pesticide while ensuring the lowest
435 possible toxicity in the treated water matrix, this could enlarge the necessary ozone
436 dosage to prohibitive values, economically speaking. Moreover, because of ACMP is
437 resistant to molecular ozone oxidation, the degradation through hydroxyl radicals will
438 be the main removal mechanism of this priority pesticide during ozonation. Therefore,
439 the water matrix characteristics will play a decisive role in the ACMP degradation
440 efficiency. In addition to the conditions that naturally favor the ozone decomposition
441 process to hydroxyl radicals, like neutral and alkaline pH conditions, strategies aimed to
442 further promote the indirect pathway should be equally investigated and employed.
443 This, of course, would be essential in order to enhance the degradation efficiency and
444 consequently reducing the oxidant dose to be applied.

445

446 **Conclusions**

447

448 The kinetics, reaction pathways and toxicity evolution during ACMP ozonation process
449 were explored for the first time. The second-order kinetic constant for the reactions of
450 ACMP with molecular ozone and hydroxyl radicals were determined to be 0.25 M⁻¹s⁻¹
451 and 2.1·10⁹ M⁻¹s⁻¹, thus clearly indicating the resistance of the pesticide structure

452 towards O₃ attack. This ozone-recalcitrance was confirmed through degradation
453 experiments at neutral pH, in which the direct reaction was barely observed. Formed
454 hydroxyl radicals showed to completely remove ACMP (initial concentration 10 μM)
455 with an ozone dosage of 5.5 mg L⁻¹, while their major intermediate products needed
456 higher doses. The proposed ACMP degradation pathways consisted of combinations of
457 oxidation and hydrolysis steps, which would yield different TPs depending on the initial
458 site in which the hydrogen abstraction by hydroxyl radicals took place. Toxicity of the
459 reaction medium increased to reach a maximum, and then decreased to relatively low
460 values. Since these changes could not be related to the single presence of some of the
461 detected TPs, they were attributed to synergistic effects among different species as well
462 as to the presence, although not identified, of intermediate aldehydes which even at very
463 low concentrations, exhibited acute toxicity to bacteria. In the view of the obtained
464 results, further promoting ozone decomposition to hydroxyl radicals appears to be
465 necessary to achieve a complete ACMP and associated toxicity abatement while
466 maintaining a reasonable efficiency.

467

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469

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474

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